

AD \_\_\_\_\_

Award Number: DAMD17-99-D-0005

TITLE: Preparation of Chemicals and Bulk Drug Substances for the  
U.S. Army Drug Development Program

PRINCIPAL INVESTIGATOR: Jaroslav Novotny, Ph.D.

CONTRACTING ORGANIZATION: Starks Associates, Incorporated  
Buffalo, New York 14213

REPORT DATE: March 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The views, opinions and/or findings contained in this report are  
those of the author(s) and should not be construed as an official  
Department of the Army position, policy or decision unless so  
designated by other documentation.

20001005 055

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE March 2000	3. REPORT TYPE AND DATES COVERED Annual (15 Feb 99 - 14 Feb 00)
----------------------------------	------------------------------	--

4. TITLE AND SUBTITLE Preparation of Chemicals and Bulk Drug Substances for the U.S. Army Drug Development Program	5. FUNDING NUMBERS DAMD17-99-D-0005
---	--

6. AUTHOR(S) Jaroslav Novotny, Ph.D.	
---	--

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Starks Associates, Incorporated Buffalo, New York 14213	8. PERFORMING ORGANIZATION REPORT NUMBER
--	--

9. E-MAIL: david.starks@mixcom.com	
---------------------------------------	--

11. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
--	--

1. SUPPLEMENTARY NOTES
------------------------

2a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited	12b. DISTRIBUTION CODE
--	------------------------

3. ABSTRACT (Maximum 200 Words)  Three task order assignments were completed during the reporting period. The first assignment was the synthesis of additional $\beta$ -artelinic acid. The second was synthesis of series of oxamic acids, the third the preparation of three heterocycles.  The following target compounds were synthesized during this period. $\beta$ -artelinic acid; $\alpha$ -artelinic acid; 1-piperidineacetic acid, .alpha.-oxo-; 1H-azepine-1-glyoxylic acid, hexahydro-; 1-pyrrolidineacetic acid, .alpha.-oxo-; acetic acid, [(1-methylethyl)amino]oxo-; acetic acid, oxo(2-propenylamino)-; acetic acid, (cyclopropylamino)oxo-; acetic acid, aminooxo-; acetic acid, (methylamino)oxo-; acetic acid, (ethylamino)oxo-; acetic acid, oxo[(2,2,2-trifluoroethyl)amino]-; acetic acid, [(bicyclo-[2.2.1]hept-2-yl)amino]oxo-, endo-; acetic acid, (methoxyamino)oxo-; acetic acid, oxo(phenylamino)-; acetic acid, oxo(2-pyridinylamino)-; 1-azetidineacetic acid, .alpha.-oxo-; pentanoic acid, 2,4-dioxo-; benzofuro[3,2-b]quinoline, 11-chloro-; [1]benzothieno[3,2-b]quinoline, 11-chloro-; 10H-quindolinium, 11-(methoxycarbonyl)-5,10-dimethyl-, iodide.	
--	--

4. SUBJECT TERMS $\beta$ -Artelinic acid (cGMP); oxamic acids; benzoquinolines, 10H-quindolinium iodide.		15. NUMBER OF PAGES 117
		16. PRICE CODE

7. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited
--	--	---	---

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## Table of Contents


	Page
Cover.....	I
SF298.....	II
Table of Contents.....	III
Foreword.....	IV
Introduction.....	1
Body.....	2
Key Research Accomplishments.....	96
Conclusions.....	103
References.....	110
Appendices.....	112

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

 Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

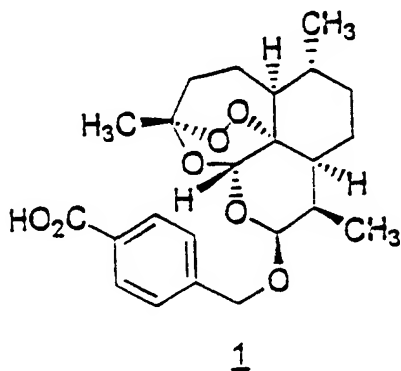
N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

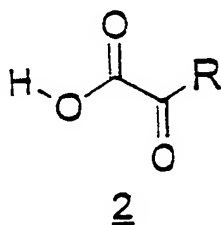
 21 Feb. 00  
PI - Signature Date

## Introduction

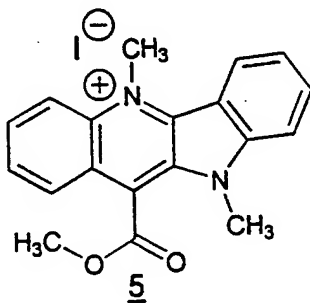
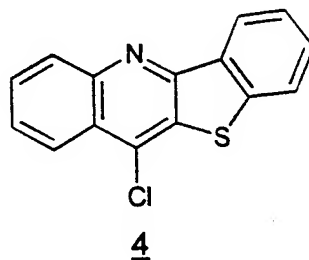
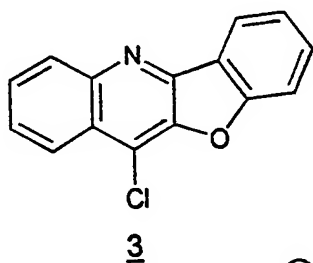
The first task order assignment of this contract (DAMD17-99-D-0005) was the preparation of additional  $\beta$ -artelinic acid (1) by cGMP. The isomeric  $\alpha$ -artelinic acid (obtained as a byproduct) was also isolated and submitted to WRAIR.



The second task order assignment consisted of the synthesis of oxamic acid 2 where R generally is the residue of a primary or secondary amine.



The third task order consisted of the synthesis of three heterocycles shown below (3-5).



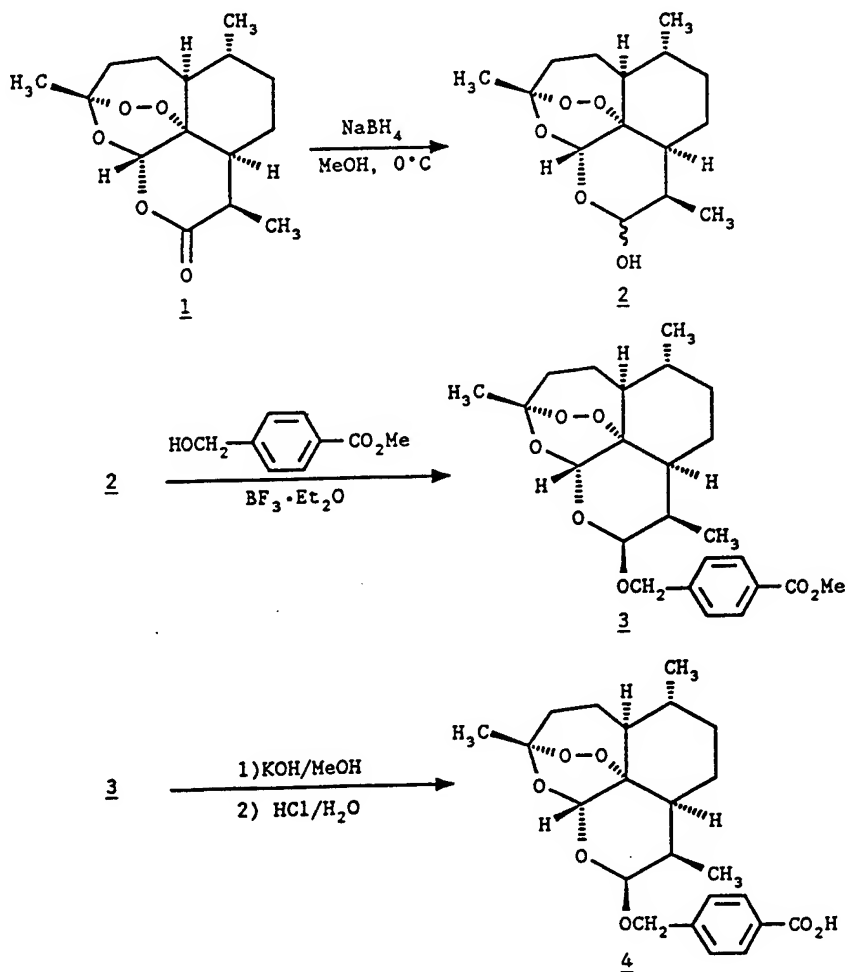
# Body

RESEARCH AND KNOWN TARGET COMPOUNDS COMPLETED AND DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH FROM FEBRUARY 15, 1999 TO FEBRUARY 14, 2000

## 1. $\beta$ -Artelinic acid (cGMP) (4)

The target compound 4 has been prepared by the following sequence of reactions.

### Reaction Sequence:



## Experimental

### Dihydroartemisinin (2)

This material (2001.6 g) was prepared by the method described in Starks Associates, Inc., Quarterly Progress Report #135, covering the period December 1, 1998 to February 28, 1999, Contract DAMD17-93-C-3003, p. 35.

### Methyl 4-(10'-dihydroartemisininoxymethyl)benzoate (3)

A portion of the pure  $\beta$ -anomer (543.6 g) and a mixture of  $\alpha$  and  $\beta$  anomers (2284.0 g) was prepared by the method described in Starks Associates, Inc., Quarterly Progress Report # 135, covering the period December 1, 1998 to February 28, 1999, Contract DAMD17-93-C-3003, pp 35-37. A portion of the mixture (1114.4 g) containing less than 2.1% of  $\alpha$ -anomer was rechromatographed on two 3 kg columns (both columns were used four times without the change of  $\text{SiO}_2$ ) to give 875.1 g of  $\beta$ -anomer and 188.4 g of a mixture of anomers. The mixture was rechromatographed on the same column to give 149.7 g of  $\beta$ -anomer and 28.2 g of a mixture. Another batch of the mixture (115.0 g) was rechromatographed on the same column to give 46.4 g of  $\beta$ -anomer and 50.6 g of a mixture. A portion of the crude mixture (467.0 g) was rechromatographed on a 22 kg  $\text{SiO}_2$  column using hexane-EtOAc (4:1) as the eluent. The fractions of pure  $\beta$ -anomer were concentrated to give 241.1 g of oil. Fractions containing a mixture of anomers gave 165.4 g of oil. One more portion of the mixture (440.9 g) was rechromatographed on the same column (without the change of  $\text{SiO}_2$ ) to give 267.6 g of  $\beta$ -anomer and 156.3 g of a mixture of anomers. The mixtures (165.4 g, 146.7 g, 28.2 g and 50.6 g) were rechromatographed on the same column (without the change of  $\text{SiO}_2$ ) to give 191.9 g of  $\beta$ -anomer and 167.0 g of a mixture. The mixtures (167.0 g and 156.3 g) were

rechromatographed on the same column (without the change of  $\text{SiO}_2$ ) to give 153.4 g of  $\beta$ -anomer and 100 g of a mixture. The mixture (100 g) was dissolved in hexane-EtOAc (4:1) (125 mL). After standing for 1 h a crystalline material separated. This was collected on a filter, washed with hexane then air-dried (27 g of  $\alpha$ -anomer). The filtrate was chromatographed on a 3 kg column to give 7.0 g of  $\beta$ -anomer and 58.1 g of a mixture. The mixture was dissolved in hexane-EtOAc (4:1) (65 mL), cooled, and the solid that separated was collected, washed with hexane (2 x 115 mL), then dried to give 11.4 g of crude  $\alpha$ -anomer. The filtrate was rechromatographed to give 5.1 g of  $\beta$ -anomer and 34.9 g of a mixture. The last two materials (5.1 g and 34.9 g) were stored.

#### Spectral Data

##### Nuclear Magnetic Resonance ( $\text{CDCl}_3$ )

$\delta$  8.01 (d, 2,  $J = 8.2$  Hz, aromatic H's at  $\text{C}_2$  and  $\text{C}_3$ );  
7.38 (d, 2,  $J = 8.3$  Hz, aromatic H's at  $\text{C}_3$  and  $\text{C}_5$ );  
5.45 (s, 1, H at  $\text{C}_{12}$ ); 4.95 (d, 1,  $J = 13.7$  Hz,  $\text{OCH}_2$ );  
4.91 (d, 1,  $J = 3.4$  Hz, H at  $\text{C}_{10}$ ); 4.58 (d, 1,  $J = 13.1$  Hz,  $\text{OCH}_2$ ); 3.91 (s, 3,  $\text{OCH}_3$ ); 1.45 (s, 3,  $\text{CH}_3$ ); 0.96 (d, 3,  $\text{CH}_3$  at  $\text{C}_6$ ); 0.95 (d, 3,  $\text{CH}_3$  at  $\text{C}_9$ ).

#### HPLC

10  $\mu\text{L}$  of 4 in  $\text{CH}_3\text{OH}$

Mobile phase:  $\text{CH}_3\text{OH} - 0.1\text{M HCO}_2\text{NH}_4$  (75:25)

Flow rate: 1 mL/min.

Column: U-Bondapak

Detector: 235 nm

HPLC indicated less than 1% of the  $\alpha$ -anomer.



### Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,  
0.25 mm silica gel 60F-254; detection - UV light.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	Hexane-EtOAc (2:1)	0.45	Homogeneous
2.	Hexane-EtOAc (4:1)	0.19	Homogeneous

### $\beta$ -Artelinic acid (4)

A solution of KOH (369 g of 85%, 5.59 mol) in  $\text{CH}_3\text{OH}$  (6.6 L) was added to the ester 3 (543.6 g, 1.257 mol) and the solution was stirred at RT for 7 days. The progress of the reaction was followed by TLC. The solution was acidified with acetic acid (875 mL) then concentrated in vacuo to a solid. The solid was partitioned between ether (12 L) and water (12 L). The organic portion was washed with  $\text{H}_2\text{O}$  (2 x 6 L), dried ( $\text{Na}_2\text{SO}_4$ ), then concentrated in vacuo to a white solid (464.1 g, 88.2 %). Additional product (1701.0 g) was obtained from three additional reactions.

The first lot (464.1 g) was crystallized from EtOH (1850 mL) and sterile water (464 mL) to give 399.1 g of 4 as the first crop and 27.7 g of 4 as the second crop. The remainder of the crude material (1701.0 g) was recrystallized in three lots from 3160 mL, 2060 mL and 1640 mL of EtOH and 777 mL, 521 mL and 410 mL of sterile  $\text{H}_2\text{O}$  respectively, to give 721.2 g, 467.4 g and 394.2 g of 4 as the first crop and 46.3 g, 37.7 g and 11.1 g of 4 as the second crop. HPLC's indicated the first crops to contain >99.5%  $\beta$ -anomer and the second crops to contain >99% of  $\beta$ -anomer. The first crops were combined (1981.9 g) then dissolved in 7927 mL of ethanol by warming to 60°C. The solution was filtered by gravity through a Schleicher & Schnell filter paper grade 582 into a 12 L flask. The solution was diluted with sterile water (1982 mL)

and the white suspension was allowed to cool to RT overnight, then cooled in an ice bath (3°C). The solid was collected on Sharkskin filter paper, washed with EtOH:H<sub>2</sub>O (4:1) (2x500 mL) then dried in vacuo to constant weight at 40°C; yield 1881.3 g (94.2% recovery). The HPLC indicated the presence of 0% α-anomer. The material was passed through a 20 mesh standard screen to give 1874.0 g of 4; mp 114-116°C. A portion (1871 g) was transmitted to WRAIR on June 7, 1999 (Lot No. 1N1-38-1).

### Analysis

	<u>C</u>	<u>H</u>
Calc'd for C <sub>23</sub> H <sub>30</sub> O <sub>7</sub> •0.5 H <sub>2</sub> O	64.62	7.31
Found	64.71, 64.62	7.39, 7.30

### Spectral Data

#### Infrared (Nujol)

Major bands: 3380, 2900, 2580, 2440, 1695, 1645, 1600, 1565, 1500, 1450, 1385, 1370, 1300, 1270, 1240, 1220, 1170, 1150, 1110, 1090, 1000, 970, 930, 860, 835, 815, 740 cm<sup>-1</sup>.

#### Ultraviolet (Ethanol)

λ<sub>max</sub> 201 nm (log ε 4.34); 235 nm (4.16).

#### Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

δ 8.08 (d, 2, J=8.1 Hz, H's at C<sub>2</sub> and C<sub>6</sub>); 7.41 (d, 2, J= 8.1 Hz, H's at C<sub>3</sub> and C<sub>5</sub>); 5.45 (s, 1, H at C<sub>12</sub>); 4.98 (d, 1, J=13.2 Hz, CH<sub>2</sub>); 4.93 (d, 1, J=3.3 Hz, H at C<sub>10</sub>); 4.59 (d, 1, J=13.2 Hz, CH<sub>2</sub>); 2.69 (m, 1, H at C<sub>9</sub>); 1.45 (s, 3, CH<sub>3</sub> at C<sub>3</sub>); 0.97 (d, 3, J=7.3 Hz, CH<sub>3</sub> at C<sub>6</sub>); 0.94 (d, 3, J=5.9 Hz, CH<sub>3</sub> at C<sub>9</sub>).

# Optical Rotation

$[\alpha]_D$  +90.34° (1.044 g/100 mL, chloroform)

## HPLC

10  $\mu$ L of 4 in CH<sub>3</sub>OH

Mobile phase: CH<sub>3</sub>OH - 0.1 M HCO<sub>2</sub>NH<sub>4</sub> (62:38)

Flow rate: 1 mL/min

Column: U-Bondapack

Detector: 235 nm

HPLC indicated no detectable amount of  $\alpha$ -anomer present.

## Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm,  
0.25 mm silica gel 60F-254; detection - UV light.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1. CH <sub>2</sub> Cl <sub>2</sub> -CH <sub>3</sub> OH (9:1)	0.51	Homogeneous

## Source of Materials

<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
Artemisinin	National Ctr. for Science & Techn.	SKN619	NJ17-31-1
"	"	NC378	NJ17-70-1
"	"	SKS379	NJ17-15-1
NaBH <sub>4</sub>	Aldrich Chemical Co.	LS01015HS	NJ17-68-1
CH <sub>3</sub> OH	J.T. Baker Chemical Co.	M28A25	NJ17-60-1
"	"	M36A07	NJ17-85-1
"	"	M06811	NJ17-49-1
AcOH	"	M03817	NJ17-82-1
"	"	M03817	NJ17-87-1
"	"	M03817	NJ17-97-1
"	"	M03817	NJ17-118-1

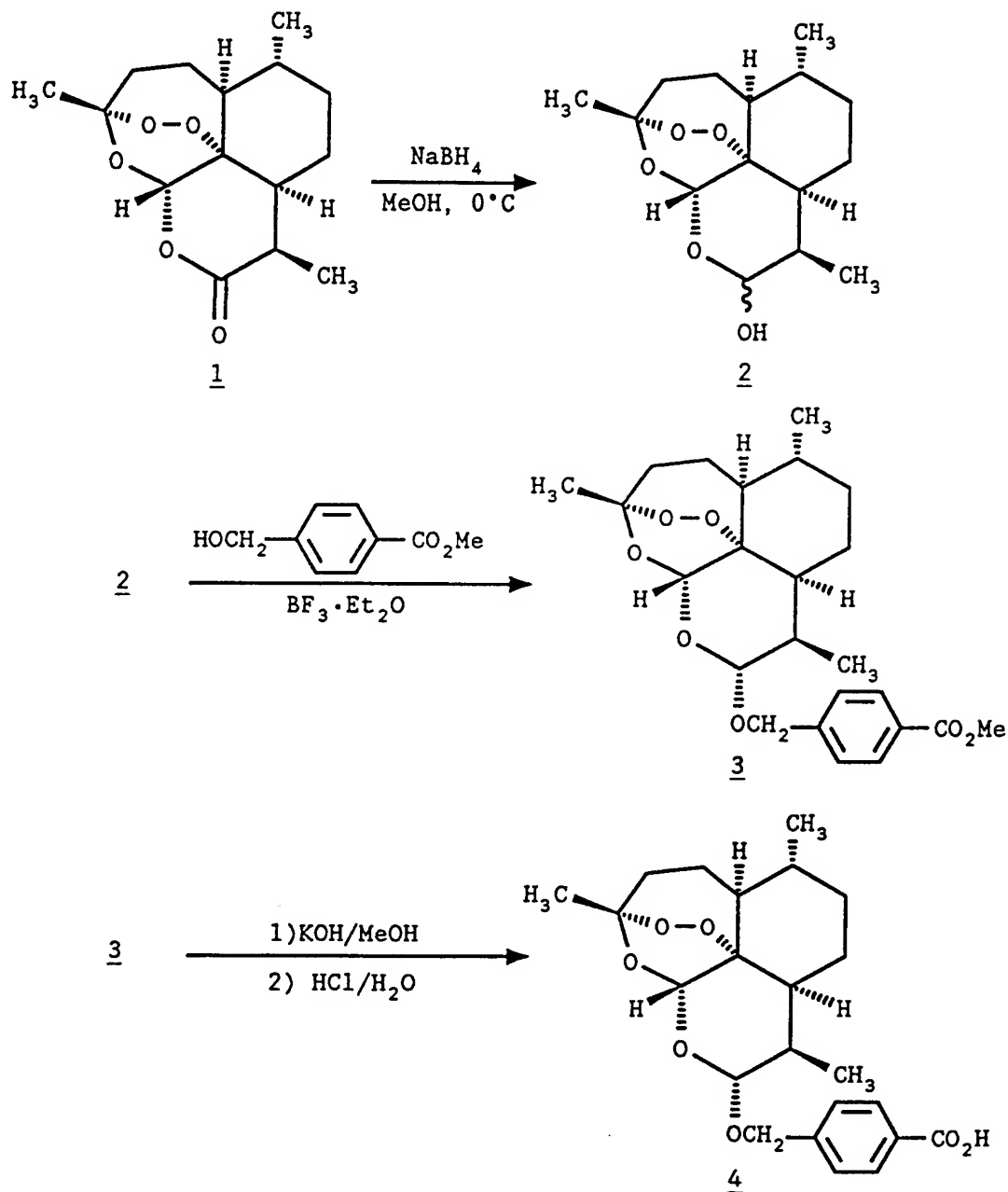
<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
Distilled H <sub>2</sub> O	Mayer Bros.		NJ17-50-1
"	"		NJ17-65-1
"	"		NJ17-80-1
"	"		NJ17-91-1
Methyl 4-hydroxymethylbenzoate	Starks Assoc., Inc.	NJ30-37-2	
"	"	NJ36-4-1	
"	"	NJ36-6-1	
"	"	NJ36-10-1	
"	Apin Chemicals Ltd.	19028	NJ31-116-1
"	"	19028	NJ31-116-2
"	"	19351	NJ31-138-1
"	"	19402	NJ34-72-1
BF <sub>3</sub> ·Et <sub>2</sub> O	Aldrich Chemical Co.	06031JS	NJ17-62-1
"	"	06031JS	NJ17-66-1
Ether	Fisher Scientific	982692-15	NJ17-73-1
"	"	982784-15	NJ17-74-1
"	"	982939-15	NJ17-92-1
NaHCO <sub>3</sub>	Aldrich Chemical Co.	09810TQ	NJ17-67-1
"	"	09810TQ	NJ17-86-1
"	"	09810TQ	NJ17-108-1
Silica gel	E. Merck	TA353534	NJ17-56-1
"	"	TA420934	NJ17-75-1
"	"	TA423134	NJ17-76-1
"	"	TA460734	NJ17-77-1
"	"	TA294934	NJ17-110-1
Na <sub>2</sub> SO <sub>4</sub>	Aldrich Chemical Co.	14011 HR	NJ17-53-1
"	J.T. Baker Chemical Co.	MO5155	NJ17-57-1
"	"	M37149	N17-115-1

<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
Hexanes	J.T. Baker Chemical Co.	L13634	NJ17-17-1
"	"	M04A05	NJ17-71-1
"	"	M07A16	NJ17-78-1
"	"	M07A16	NJ17-84-1
"	"	M16A03	NJ17-88-1
"	"	M07A16	NJ17-96-1
"	"	M46A07	NJ17-109-1
"	"	M46A07	NJ17-117-1
Ethyl acetate	"	L13614	NJ17-18-1
"	"	M30A17	NJ17-72-1
"	"	M30A17	NJ17-79-1
"	"	M38A35	NJ17-95-1
"	"	M38A35	NJ17-113-1
KOH	Aldrich Chemical Co.	05128KQ	NJ17-24-1
"	"	05128KQ	NJ17-81-1
"	"	09811ES	NJ17-90-1
"	"	00424PS	NJ17-116-1
EtOH	Quantum Chem. Co.	T8FF72	NJ17-64-1
"	Aaper Chemical & Alcohol Co.	98K12QA	NJ17-94-1
"	"	99A25QA	NJ17-119-1
Sterile H <sub>2</sub> O	McGaw Inc.	J8A218	NJ17-89-1

## 2. $\alpha$ -Artelinic acid (4)

The target compound 4 has been prepared by the following sequence of reactions.

### Reaction Sequence



## Experimental

### Dihydroartemisinin (2)

To a cooled (0°C), stirred suspension of artemisinin (200 g, 0.708 mol) in CH<sub>3</sub>OH (4 L) was added in small portions, sodium borohydride (80 g, 2.115 mol), during 1 h, keeping the temperature below 0°C. The suspension was stirred below 0°C for 1.5 h or until all the starting material had reacted as shown by TLC analysis using hexane-EtOAc (4:1) as the eluting solvent. The mixture was neutralized with a solution of acetic acid (140 mL) in CH<sub>3</sub>OH (140 mL) then concentrated in vacuo to a paste (bath temperature below 20°C). The paste was suspended in water (6 L), and the suspension was stirred for 15 min. The solid was collected on a filter, washed with a mixture of H<sub>2</sub>O-CH<sub>3</sub>OH (2:1) (2 x 960 mL) then dried in vacuo at RT to constant weight; yield 187.5 g (93.1%), mp 142-144°C, lit.<sup>1a,1b</sup> mp 153-154°C. Additional product was obtained from other reactions for a total of 6430.2 g of 2 (91.7% overall). A portion (4428.6 g) was used in the synthesis of the first part of artelinic acid (3990 g), the remainder (2001.6 g) in the second.

### Spectral Data

#### Nuclear Magnetic Resonance (CDCl<sub>3</sub>)

δ 5.61 (s, 1, H at C<sub>12</sub> for β-anomer); 5.39 (s, 1, H at C<sub>12</sub> for α-anomer); 5.30 (d, 1, J= 3.3 Hz, H at C<sub>10</sub> for β-anomer); 4.74 (d, 1, J= 9.2 Hz, H at C<sub>10</sub> for α-anomer).

### Methyl 4-(10'-dihydroartemisininoxymethyl)benzoate (3)

To a stirred suspension of 2 (183 g, 0.646 mol) in ether (4 L) was added methyl 4-hydroxymethylbenzoate (160.4 g, 0.965

mol) followed by  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (104.5 g, 0.736 mol). The resulting solution was stirred at RT for 24 h, washed with 5%  $\text{NaHCO}_3$  solution (2 x 2.1 L),  $\text{H}_2\text{O}$  (2 x 2.1 L), dried ( $\text{Na}_2\text{SO}_4$ , 400 g) then concentrated in vacuo to an oil (324.2 g). This was chromatographed on a column of  $\text{SiO}_2$  (3 kg) using hexane-EtOAc (4:1, 25 L) as the eluent. Fractions containing pure  $\beta$ -anomer were concentrated to give 25.5 g of oil. Fractions containing a mixture of  $\beta$ -anomer and  $\alpha$ -anomer (8 L) were combined then concentrated to an oil (235.3 g). Additional pure  $\beta$ -anomer (473.7 g), and an additional mixture of  $\beta$  and  $\alpha$  anomers (2220.9 g), was obtained from other reactions. A portion of the mixture (487.3 g) was rechromatographed on a 22 kg  $\text{SiO}_2$  column using hexane-EtOAc (4:1) as the eluent. Fractions containing pure  $\beta$ -anomer were concentrated to give 268.6 g of oil. Fractions containing a mixture of anomers gave 165.0 g of oil. Three more portions of the mixture (479.6 g, 488.3 g, and 413.4 g) were rechromatographed on the same column (without the change of  $\text{SiO}_2$ ) to give 281.6 g, 251.8 g and 204.9 g of  $\beta$ -anomer and 135.0 g, 187.0 g and 211.0 g of a mixture of anomers. The mixtures (165.0 g, 135.0 g, 187.0 g and 211.0 g) were rechromatographed on the same column (without the change of  $\text{SiO}_2$ ) to give 149.8 g of pure  $\beta$ -anomer and 467.0 g of a mixture. Total yield of  $\beta$ -anomer from one column, 1156.7 g. Two additional columns yielded 3935.3 g of pure  $\beta$ -anomer and 115 g of a mixture. Fractions containing mainly  $\alpha$ -anomer from nine 22 kg columns were combined then concentrated in vacuo to yield 308.7 g of crude  $\alpha$ -anomer. This was combined with 11.4 g of crude  $\alpha$ -anomer obtained earlier, then recrystallized from hexane (8333 mL); yield 206.8 g (67.0% recovery), mp 109-110°C. Additional product of similar purity (56.5 g) was obtained from mother liquor. Fractions containing mainly  $\alpha$ -anomer from nine 22 kg columns were combined then concentrated in vacuo to yield 335 g of crude product. The solid was recrystallized from hexane (4 L), clarified by filtration and allowed to stand at RT for 5.5 days. The precipitate was collected, washed with



hexanes (2 x 1.5 L), then dried in vacuo at RT to constant weight (220.8 g).

### Spectral Data

#### Nuclear Magnetic Resonance (CDCl<sub>3</sub>)

δ 8.00 (d, 2, J=8.2 Hz, aromatic H's at C<sub>2</sub> and C<sub>6</sub>);  
7.43 (d, 2, J= 8.1 Hz, aromatic H's at C<sub>3</sub> and C<sub>5</sub>);  
5.34 (s, 1, H at C<sub>12</sub>); 5.03 (d, 1, J=13.2 Hz, OCH<sub>2</sub>);  
4.68 (d, 1, J=13.2 Hz, OCH<sub>2</sub>); 4.52 (d, 1, J=9.2 Hz,  
H at C<sub>10</sub>); 3.91 (s, 3, OCH<sub>3</sub>); 1.46 (s, 3, CH<sub>3</sub>); 0.95  
(d, 3, J=5.9 Hz, CH<sub>3</sub> at C<sub>6</sub>); 0.93 (d, 3, J=7.1 Hz,  
CH<sub>3</sub> at C<sub>9</sub>).

#### α-Artelinic acid (4)

A solution of KOH (138.0 g of 85%, 2.09 mol) in CH<sub>3</sub>OH (2510 mL) was added to the ester (3) (206.8 g, 0.478 mol) and the solution was stored for 6 days. The progress of the reaction was followed by TLC. The solution was acidified with acetic acid (330 mL) with cooling (15-24°C) then concentrated in vacuo to a solid (~675 g). The solid was partitioned between Et<sub>2</sub>O (4 L) and water (4 L). The phases were separated and the organic portion was washed with water (2 x 2 L), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated to a solid (200 g). Additional product of similar purity (274.6 g) was obtained from two additional runs. The materials were combined, dissolved in ethanol (1470 mL) by warming to 45°C, filtered, then diluted with sterile water (368 mL) and cooled in an ice bath for 2 h. The solid was collected on a filter, washed with cold EtOH:H<sub>2</sub>O (4:1) (250 mL) then dried in vacuo at 30°C to constant weight (440.8 g). The material was passed through a standard stainless steel 20 mesh screen to give 439.0 g of

pure product; m.p. 144-150°C. A portion (438.6 g) was transmitted to WRAIR in June 1, 1999 (Lot No. 1N1-32-1).

Analysis

	<u>C</u>	<u>H</u>
Calc'd for $C_{23}H_{30}O_7$	66.01	7.23
Found	66.01	7.20

Spectral Data

Infrared (Nujol)

Major bands: 3380, 2900, 2580, 2440, 1695, 1645, 1600, 1565, 1500, 1450, 1385, 1370, 1300, 1270, 1240, 1220, 1170, 1150, 1110, 1090, 1000, 970, 930, 860, 835, 815, 740  $cm^{-1}$ .

Ultraviolet (Ethanol)

$\lambda_{max}$  201 nm ( $\log \epsilon$  4.39); 235 nm (4.18).

Nuclear Magnetic Resonance (DMSO- $d_6$ )

$\delta$  8.07 (d, 2,  $J=8.1$  Hz, H's at  $C_2$  and  $C_6$ ); 7.45 (d, 2,  $J=8.4$  Hz, H's at  $C_3$  and  $C_5$ ); 5.34 (s, 1, H at  $C_{12}$ ); 5.05 (d, 1,  $J=13.6$  Hz,  $CH_2$ ); 4.69 (d, 1,  $J=13.6$  Hz,  $CH_2$ ); 4.52 (d, 1,  $J=9.2$  Hz, H at  $C_{10}$ ); 2.52 (m, 1, H at  $C_9$ ); 1.45 (s, 3,  $CH_3$  at  $C_3$ ); 0.95 (d, 3,  $J=1.8$  Hz,  $CH_3$  at  $C_8$ ); 0.93 (d, 3,  $J=3.3$  Hz,  $CH_3$  at  $C_9$ ).

HPLC

10  $\mu$ L of 4 in  $CH_3OH$

Mobile phase:  $CH_3OH$  - 0.1M  $HCO_2NH_4$  (62:38)

Flow rate: 1 mL/min.

Column: U-Bondapak

Detector: 235 nm

HPLC indicated no detectable amount of  $\beta$ -anomer present.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm,  
0.25 mm silica gel 60F-254; detection - UV light.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	CH <sub>2</sub> Cl <sub>2</sub> -MeOH (9:1)	0.38	Homogeneous

Source of Materials

<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
Artemisinin	National Ctr. for Science & Techn.	SKN619	NJ17-31-1
"	"	NC378	NJ17-70-1
"	"	SKS379	NJ17-15-1
NaBH <sub>4</sub>	Aldrich Chemical Co.	LS01015HS	NJ17-68-1
CH <sub>3</sub> OH	J.T. Baker Chemical Co.	M28A25	NJ17-60-1
"	"	M36A07	NJ17-85-1
AcOH	"	M06811	NJ17-49-1
"	"	M03817	NJ17-82-1
"	"	M03817	NJ17-87-1
"	"	M03817	NJ17-97-1
"	"	M03817	NJ17-118-1
Distilled H <sub>2</sub> O	Mayer Bros.		NJ17-50-1
"	"		NJ17-65-1
"	"		NJ17-80-1
"	"		NJ17-91-1
Methyl 4- hydroxymethyl- benzoate	Starks Assoc., Inc.	NJ30-37-2	
"	"	NJ36-4-1	
"	"	NJ36-6-1	
"	"	NJ36-10-1	
"	Apin Chemicals Ltd.	19028	NJ31-116-1
"	"	19028	NJ31-116-2
"	"	19351	NJ31-138-1
"	"	19402	NJ34-72-1

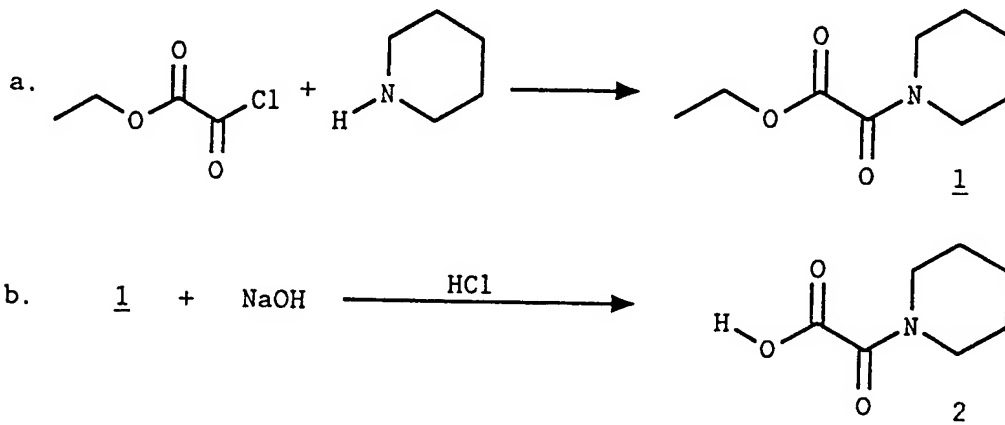
<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
BF <sub>3</sub> ·Et <sub>2</sub> O	Aldrich Chemical Co.	06031JS	NJ17-62-1
"	"	06031JS	NJ17-66-1
Ether	Fisher Scientific	982692-15	NJ17-73-1
"	"	982784-15	NJ17-74-1
"	"	982939-15	NJ17-92-1
NaHCO <sub>3</sub>	Aldrich Chemical Co.	09810TQ	NJ17-67-1
"	"	09810TQ	NJ17-86-1
"	"	09810TQ	NJ17-108-1
Silica gel	E. Merck	TA353534	NJ17-56-1
"	"	TA420934	NJ17-75-1
"	"	TA423134	NJ17-76-1
"	"	TA460734	NJ17-77-1
"	"	TA294934	NJ17-110-1
Na <sub>2</sub> SO <sub>4</sub>	Aldrich Chemical Co.	14011 HR	NJ17-53-1
"	J.T. Baker Chemical Co.	M05155	NJ17-57-1
"	"	M37149	N17-115-1
Hexanes	J.T. Baker Chemical Co.	L13634	NJ17-17-1
"	"	M04A05	NJ17-71-1
"	"	M07A16	NJ17-78-1
"	"	M07A16	NJ17-84-1
"	"	M16A03	NJ17-88-1
"	"	M07A16	NJ17-96-1
"	"	M46A07	NJ17-109-1
"	"	M46A07	NJ17-117-1
Ethyl acetate	"	L13614	NJ17-18-1
"	"	M30A17	NJ17-72-1
"	"	M30A17	NJ17-79-1
"	"	M38A35	NJ17-95-1
"	"	M38A35	NJ17-113-1

<u>Compound</u>	<u>Vendor</u>	<u>Lot No.</u>	<u>Internal Control No.</u>
KOH	Aldrich Chemical Co.	05128KQ	NJ17-24-1
"	"	05128KQ	NJ17-81-1
"	"	09811ES	NJ17-90-1
"	"	00424PS	NJ17-116-1
EtOH	Quantum Chem. Co.	T8FF72	NJ17-64-1
"	Aaper Chemical & Alcohol Co.	98K12QA	NJ17-94-1
"	"	99A25QA	NJ17-119-1
Sterile H <sub>2</sub> O	McGaw Inc.	J8A218	NJ17-89-1

3. 1-Piperidineacetic acid, .alpha.-oxo- (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

1-Piperidineacetic acid, .alpha.-oxo-, ethyl ester (1)

To a cold (0°), stirred solution of ethyl oxalyl chloride (19.2 g, 0.141 mol) in toluene (50 mL) was added a solution of piperidine (10.0 g, 0.117 mol) and pyridine (18.5 g, 0.234 mol) in toluene (50 mL), dropwise, while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 0.5 h. Methanol (25 mL) was slowly added to the reaction mixture, and the resulting solution was concentrated in vacuo to a solid residue. The residue was extracted with EtOAc (3 x 50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then

concentrated to a volume of ~25 mL. The solid that separated was collected, then dried; yield 23.4 g (>100%). The material was purified by chromatography on a column of SiO<sub>2</sub> (100 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were combined then concentrated in vacuo to give 19.9 g (91.8%) of pure 1. The material was suitable for further transformation.

1-Piperidineacetic acid, .alpha.-oxo- (2)<sup>3</sup>

To a cold (0°C), stirred solution of 1 (10.0 g, 0.054 mol) in CH<sub>3</sub>OH (50 mL) was added a solution of NaOH (2.2 g, 0.055 mol) in H<sub>2</sub>O (10 mL), dropwise, over 10 min. The mixture was allowed to warm to room temperature then stirred for 1 h. TLC (hexane-EtOAc 1:1) showed no starting material. The mixture was concentrated to remove MeOH, and to the slurry was added H<sub>2</sub>O (20 mL). The solution was cooled in an ice bath to 10°C then acidified with a solution of HCl (5.5 mL) in H<sub>2</sub>O (20 mL). The acid solution was extracted with EtOAc (4 x 50 mL). The combined organic extracts were washed with H<sub>2</sub>O (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a solid; yield 5.7 g (67.1%). The material was crystallized from hexane-EtOAc (3:1) (80 mL) to give 4.4 g of 2. This was recrystallized from EtOAc-hexane (2:1) (30 mL) to give 2.0 g of 2. This material was combined with product obtained from the mother liquor of the second crystallization then recrystallized from EtOAc-hexane (5:1) (130 mL); yield 2.5 g, mp 124-124.5°C, literature<sup>4</sup> mp 128-129°C. A portion (2.1 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-5-5).



Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>7</sub> H <sub>11</sub> NO <sub>3</sub>	53.49	7.05	8.91
Found	53.62	6.99	8.85

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  2958, 2877, 2747, 2671, 2552, 2481, 1744, 1606, 1502, 1473, 1449, 1410, 1369, 1292, 1241, 1215, 1140, 1125, 1015, 956, 927, 895, 865, 836, 813, 801, 747, 710, 688, 657, 555, 542, 470, 441, 422 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H  $\delta$  10.74 (s, 1, CO<sub>2</sub>H); 3.74 (d, 2, J=5.5 Hz, 1 each at C-2 and C-6); 3.61 (d, 2, J=5.5 Hz, 1H each at C-2 and C-6); 1.66 (d, 6, H's at C-3, C-4 and C-5).

<sup>13</sup>C  $\delta$  162.1, 160.6, 47.8, 43.6, 26.2, 25.2, 24.1.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254, 0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.70
2.	n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.27

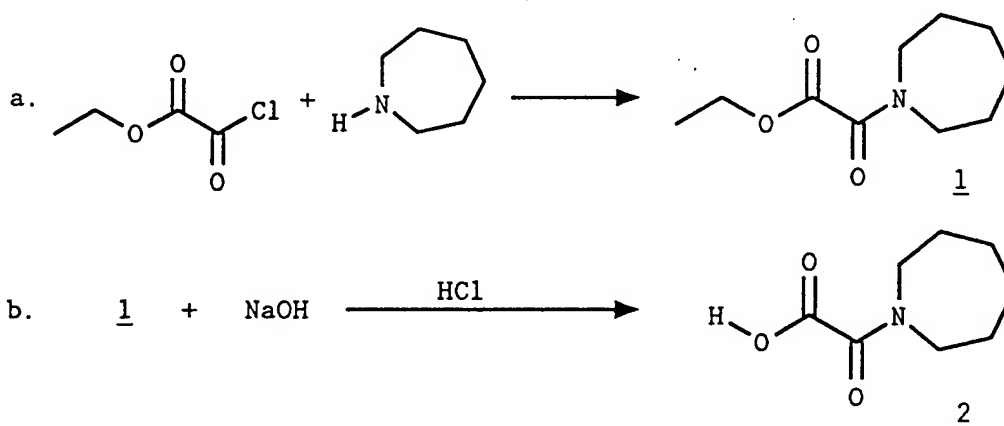
Source of Materials

1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. Toluene	Aldrich Chemical Co., Inc.
3. Pyridine	Aldrich Chemical Co., Inc.
4. Piperidine	Aldrich Chemical Co., Inc.
5. Methanol	J.T. Baker Chemical Co.
6. Ethyl acetate	J.T. Baker Chemical Co.
7. Silica gel	Merck & Co, Inc.
8. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.

4. 1H-Azepine-1-glyoxylic acid, hexahydro- (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

1H-Azepine-1-glyoxylic acid, hexahydro-, ethyl ester (1)

To a cold (0°), stirred solution of ethyl oxalyl chloride (16.5 g, 0.121 mol) in toluene (50 mL) was added a solution of hexamethyleneimine (10.0 g, 0.101 mol) and pyridine (15.8 g, 0.200 mol) in toluene (50 mL), dropwise, while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 0.5 h. Methanol (25 mL) was slowly added to the reaction mixture, and the resulting solution was concentrated in vacuo to a solid residue. The residue was

extracted with EtOAc (3 x 50 mL). The combined extracts were washed with H<sub>2</sub>O (3 x 25 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated in vacuo to an oil (21.5 g, >100%). The material was purified by chromatography on a column of SiO<sub>2</sub> (100 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were combined then concentrated in vacuo to give 19.5 g (98.0%) of pure 1. The material was suitable for further transformation.

1H-Azepine-1-glyoxylic acid, hexahydro- (2)

To a cold (0°C), stirred solution of 1 (10.0 g, 0.050 mol) in EtOH (50 mL) was added a solution of NaOH (2.1 g, 0.052 mol) in H<sub>2</sub>O (10 mL), dropwise, over 10 min. The mixture was allowed to warm to room temperature then stirred for 1 h. The mixture was concentrated in vacuo to remove EtOH, and to the slurry was added H<sub>2</sub>O (20 mL). The solution was cooled in an ice bath to 10°C then acidified with a solution of HCl (5 mL) in H<sub>2</sub>O (20 mL). The acid solution was extracted with EtOAc (4 x 50 mL), and the extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a solid. The material was crystallized from EtOAc-hexane (5:1) (30 mL) to give 3.8 g of pure 2, mp 125-125.5°C. A portion (3.4 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-12-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>8</sub> H <sub>13</sub> NO <sub>3</sub>	56.13	7.65	8.18
Found	56.14	7.61	8.19

## Spectral Data

### FT-Infrared (KBr pellet)

$\nu$  3448, 2930, 2854, 2459, 1985, 1743,  
1598, 1499, 1445, 1406, 1376, 1352, 1313,  
1289, 1250, 1201, 1165, 1106, 1000, 977,  
923, 886, 845, 796, 713, 655, 531, 503, 438  
 $\text{cm}^{-1}$ .

### Nuclear Magnetic Resonance (500 MHz, $\text{CDCl}_3$ )

$^1\text{H}$   $\delta$  9.04 (s, 1,  $\text{CO}_2\text{H}$ ); 3.85 (t, 2,  $J=6.0$  Hz,  
1H each at C-2 and C-7); 3.61 (t, 2,  $J=6.1$   
Hz, 1H each at C-2 and C-7); 1.81 (dd, 4,  
 $J=10.7$  Hz, 3.6 Hz, H's at C-3 and C-6); 1.61  
(m, 4 H's at C-4 and C-5).

$^{13}\text{C}$   $\delta$  161.6, 49.1, 47.4, 29.0, 27.1, 26.5.

### Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	$\text{CH}_3\text{OH}-\text{NH}_4\text{OH}-\text{CHCl}_3$ (1:1:6)	0.70
2.	$n\text{-BuOH}-\text{AcOH}-\text{H}_2\text{O}$ (5:1:2)	0.32

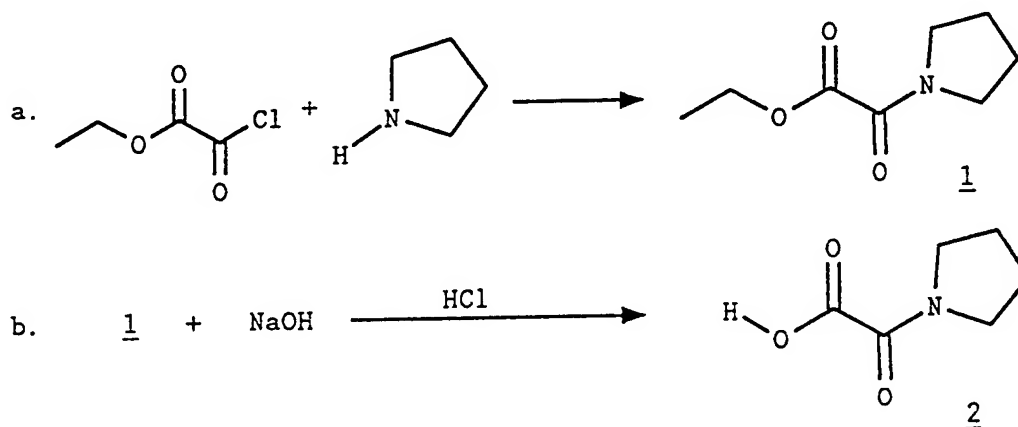
Source of Materials

1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. Toluene	Aldrich Chemical Co., Inc.
3. Pyridine	Aldrich Chemical Co., Inc.
4. Hexamethyleneimine	Aldrich Chemical Co., Inc.
5. Methanol	J.T. Baker Chemical Co.
6. Ethyl acetate	J.T. Baker Chemical Co.
7. Silica gel	Merck & Co., Inc.
8. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.
11. EtOH	Aaper Alcohol & Chemical Co.

5. 1-Pyrrolidineacetic acid, .alpha.-oxo- (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

1-Pyrrolidineacetic acid, .alpha.-oxo-, ethyl ester (1)

To a cold (0°C), stirred solution of ethyl oxalyl chloride (23.2 g, 0.170 mol) in toluene (50 mL) was added a solution of pyrrolidine (10.0 g, 0.141 mol) and pyridine (22.1 g, 0.279 mol) in toluene (50 mL), while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 0.5 h. Methanol (25 mL) was slowly added to the reaction mixture, and the resulting solution was concentrated in vacuo to a solid residue. The residue was extracted with

EtOAc (3 x 50 mL), and the combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a yellow oil (27.6 g, >100%). The material was purified by chromatography on a column of SiO<sub>2</sub> (100 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were combined then concentrated in vacuo to give 19.1 g (79.7%) of pure 1. The material was suitable for further transformation.

1-Pyrrolidineacetic acid, .alpha.-oxo- (2)

To a cold (0°C), stirred solution of 1 (10.0 g, 0.058 mol) in CH<sub>3</sub>OH (50 mL) was added a solution of NaOH (2.4 g, 0.060 mol) in H<sub>2</sub>O (10 mL), dropwise, over 10 min. The mixture was allowed to warm to room temperature then stirred for 1 h. TLC (hexane-EtOAc 1:1) indicated no starting material. The mixture was concentrated in vacuo to remove CH<sub>3</sub>OH, and to the slurry was added H<sub>2</sub>O (20 mL). The solution was cooled in an ice bath and acidified with a solution of conc. HCl (6.0 mL) in H<sub>2</sub>O (20 mL). The acid solution was extracted with EtOAc (4 x 50 mL) and the combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a solid (3.1 g, 37.3%). The material was recrystallized from EtOAc (50 mL) to give 2 (2.2 g, 71% recovery). A similar reaction starting with 8.5 g of 1 gave 1.5 g of 2. The two lots were combined then recrystallized from EtOAc (75 mL) to give 2.3 g of pure 2, mp 142.5-143°C. A portion (1.8 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-16-1).



Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_6H_9NO_3$	50.34	6.33	9.78
Found	50.57	6.33	9.66

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  990, 2897, 2778, 2720, 2472, 2367,  
1911, 1742, 1607, 1461, 1423, 1337,  
1263, 1190, 1175, 1159, 982, 948, 917,  
867, 844, 813, 763, 693, 512, 428  $cm^{-1}$ .

Nuclear Magnetic Resonance (500 MHz,  $CDCl_3$ )

$^1H$   $\delta$  4.00 (t, 2,  $J=7.0$  Hz, 1H each at C-2 and C-5); 3.61 (t, 2,  $J=7.0$  Hz, 1H each at C-2 and C-5); 2.02 (p, 2,  $J=7.1$  Hz, 1H each at C-3 and C-4); 1.92 (p, 2,  $J=6.9$  Hz, 1H each at C-3 and C-4).

$^{13}C$   $\delta$  159.2, 156.7, 49.2, 48.3, 26.5, 23.6.

### Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254, 0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>R<sub>f</sub></u>
1.	CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.67
2.	<i>n</i> -BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.23

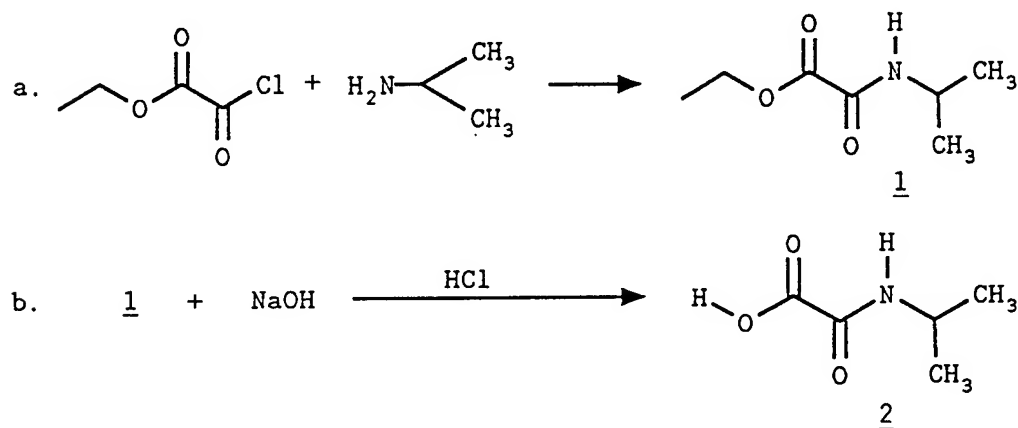
### Source of Materials

1.	Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2.	Toluene	Aldrich Chemical Co., Inc.
3.	Pyridine	Aldrich Chemical Co., Inc.
4.	Pyrrolidine	Aldrich Chemical Co., Inc.
5.	Methanol	J.T. Baker Chemical Co.
6.	Ethyl acetate	J.T. Baker Chemical Co.
7.	Silica gel	Merck & Co., Inc.
8.	Hexane	J.T. Baker Chemical Co.
9.	NaOH	J.T. Baker Chemical Co.
10.	HCl	J.T. Baker Chemical Co.

6. Acetic acid, [(1-methylethyl)amino]oxo- (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Acetic acid, [(1-methylethyl)amino]oxo-, ethyl ester (1)

To a cold (0°), stirred solution of ethyl oxalyl chloride (22.7 g, 0.166 mol) in toluene (50 mL) was added a solution of isopropylamine (10.0 g, 0.166 mol) and pyridine (26.3 g, 0.332 mol) in toluene (50 mL), while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 0.5 h. Methanol (25 mL) was slowly added to the reaction mixture, and the resulting solution was concentrated in

vacuo to a residue. The residue was extracted with EtOAc (3 x 50 mL), and the combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated in vacuo to an oil (23.2 g, 87.9%). The material was purified by chromatography on a column of SiO<sub>2</sub> (100 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were combined then concentrated in vacuo to give 20.6 g (78.0%) of pure 1. The material was suitable for further transformation.

Acetic acid, [(1-methylethyl)amino]oxo- (2)

To a solution of 1 (10.0 g, 0.063 mol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added a solution of NaOH (2.5 g, 0.065 mol) in H<sub>2</sub>O, and the mixture was stirred vigorously for 1 h. The aqueous phase was separated, acidified with a solution of conc. HCl (6.2 mL) in H<sub>2</sub>O (10 mL), then extracted with EtOAc (3 x 50 mL). The combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a white solid (5.2 g, 63.4%). The material was crystallized from EtOAc-hexane (5:1) to give 3.0 g of pure 2, mp 115-115.5°C; literature<sup>5</sup> mp 110-113°C. A portion (2.7 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-26-2).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>5</sub> H <sub>9</sub> NO <sub>3</sub>	45.80	6.92	10.68
Found	45.73	6.91	10.70

## Spectral Data

### FT-Infrared (KBr pellet)

$\nu$  3294, 3092, 2982, 2945, 2882, 1769, 1675,  
1556, 1471, 1371, 1340, 1258, 1186, 1161,  
1135, 967, 913, 766, 715, 527, 420  $\text{cm}^{-1}$ .

### Nuclear Magnetic Resonance (500 MHz, $\text{CDCl}_3$ )

$^1\text{H}$   $\delta$  10-8 (bs,  $\text{CO}_2\text{H}$ ); 7.14 (s, 1, NH); 4.08  
(m, 1, CH); 1.26 (d, 6,  $J=6.6$  Hz, 2 x  
 $\text{CH}_3$ ).

$^{13}\text{C}$   $\delta$  160.2, 156.5, 43.2, 22.0.

## Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>Rf</u>
1. $\text{CH}_3\text{OH}-\text{NH}_4\text{OH}-\text{CHCl}_3$ (1:1:6)	0.70
2. $n\text{-BuOH}-\text{AcOH}-\text{H}_2\text{O}$ (5:1:2)	0.37

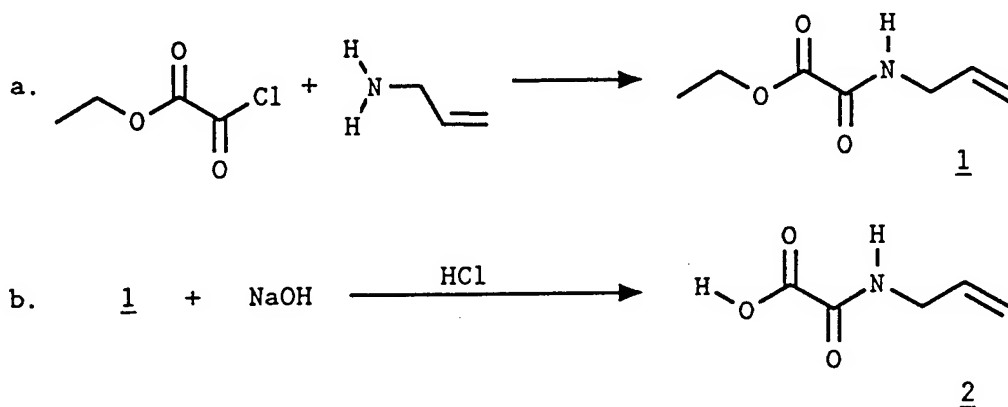
Source of Materials

1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. $\text{CH}_2\text{Cl}_2$	J.T. Baker Chemical Co.
3. Pyridine	Sigma Chemical Co.
4. Isopropylamine	Lancaster Synthesis
5. Methanol	J.T. Baker Chemical Co.
6. Ethyl acetate	J.T. Baker Chemical Co.
7. Silica gel	Merck & Co., Inc.
8. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.

7. Acetic acid, oxo(2-propenylamino) - (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Acetic acid, oxo(2-propenylamino)-, ethyl ester (1)

To a cold (0°), stirred solution of ethyl oxalyl chloride (23.4 g, 0.171 mol) in toluene (50 mL) was added a solution of allylamine (10.0 g, 0.171 mol), pyridine (27.1 g, 0.342 mol) in toluene (50 mL), dropwise while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 1 h. TLC (hexane-EtOAc 1:1) indicated a complete reaction. Methanol (25 mL) was slowly added to

the reaction mixture, and the resulting solution was concentrated in vacuo. The residue was extracted with EtOAc (3 x 50 mL), and the combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated in vacuo to an oil (22.7 g, 84.4%). The material was purified by chromatography on a column of SiO<sub>2</sub> (100 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were combined then concentrated in vacuo to give 19.0 g (70.6%) of pure 1. The material was suitable for further transformation.

Acetic acid, oxo(2-propenylamino)- (2)

To a solution of 1 (15.0 g, 0.096 mol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added a solution of NaOH (3.8 g, 0.0954 mol) in H<sub>2</sub>O (50 mL), and the mixture was stirred vigorously for 1 h. The aqueous phase was separated, acidified with a solution of conc. HCl (9.9 mL) in H<sub>2</sub>O (20 mL), then extracted with EtOAc (3 x 50 mL). The combined extracts were washed with H<sub>2</sub>O (3 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a solid (7.7 g). The material was crystallized from EtOAc-hexane (4:1) (50 mL) to give 2.8 g of pure 2, mp 98-98.5°C; literature<sup>6</sup> mp 97-98°C. A portion (2.5 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-27-2).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	46.51	5.46	10.85
Found	46.56, 46.56	5.40, 5.44	10.92, 10.90



## Spectral Data

### FT-Infrared (KBr pellet)

$\nu$  3351, 3220, 2937, 1762, 1684, 1646, 1558,  
1438, 1364, 1350, 1272, 1248, 1182, 1037,  
990, 932, 839, 806, 728, 643, 559, 524,  
488<sup>-1</sup> cm.

### Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H  $\delta$  9.45 (broad s, CO<sub>2</sub>H); 7.42 (s, 1, NH);  
5.87-5.78 (m, 1 CH=); 5.29-5.22 (m, 2,  
CH<sub>2</sub>=); 4.00-3.96 (m, 2, CH<sub>2</sub>).

<sup>13</sup>C  $\delta$  161.5, 157.6, 132.7, 116.8, 41.9.

## Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>R<sub>f</sub></u>
1. CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.69
2. n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.38

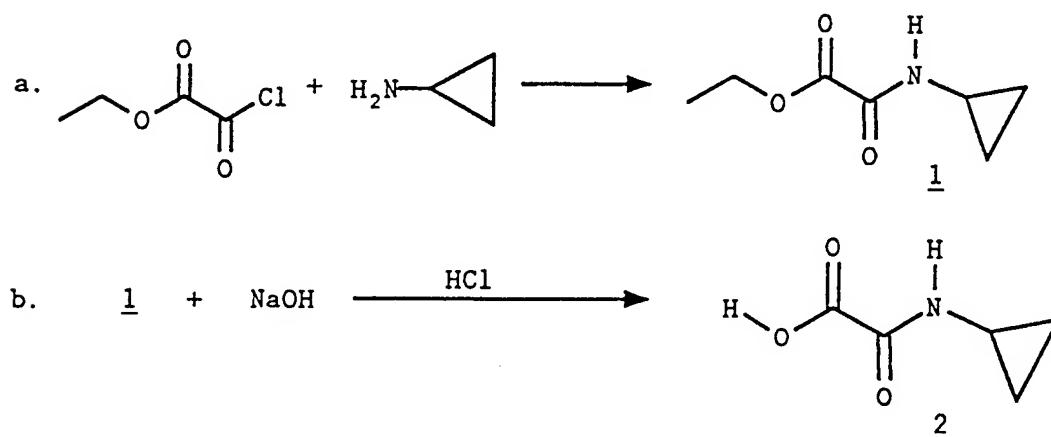
Source of Materials

1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. $\text{CH}_2\text{Cl}_2$	J.T. Baker Chemical Co.
3. Pyridine	Sigma Chemical Co.
4. Allylamine	Lancaster Synthesis
5. $\text{CH}_3\text{OH}$	J.T. Baker Chemical Co.
6. EtOAc	J.T. Baker Chemical Co.
7. Silica gel	Merck & Co., Inc.
8. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.

8. Acetic acid, (cyclopropylamino)oxo- (2)

The target compound (2) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental<sup>7</sup>

Acetic acid, (cyclopropylamino)oxo-, ethyl ester (1)

To a cold (0°), stirred solution of ethyl oxalyl chloride (20.0 g, 0.146 mol) in toluene (50 mL) was added a solution of cyclopropylamine (10.2 g, 0.175 mol) and pyridine (27.7 g, 0.350 mol) in toluene, dropwise, while keeping the reaction temperature at 0°C. The mixture was allowed to warm to room temperature then stirred for 1 h. Methanol (25 mL) was slowly added, and the resulting solution was concentrated in vacuo. The residue was extracted with EtOAc (3 x 50 mL), and the combined extracts

were washed with H<sub>2</sub>O (2 x 25 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated in vacuo (16.8 g, 73.2%). The material was suitable for further transformation.

Acetic acid, (cyclopropylamino)oxo- (2)

---

To a cold (0°C), stirred solution of 1 (6.2 g, 0.039 mol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added a solution of NaOH (1.6 g, 0.04 mol) in H<sub>2</sub>O (50 mL), and the mixture was stirred vigorously for 2 h at room temperature. The aqueous portion was acidified with a solution of conc. HCl (4.0 mL) in H<sub>2</sub>O (10 mL). The acid solution was extracted with EtOAc (3 x 50 mL), and the combined extracts were washed with H<sub>2</sub>O (2 x 25 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo to a solid (1.5 g). The material was crystallized from EtOAc to give 0.8 g of pure 2, mp 150-150.5°C; A portion (0.5 g) was transmitted to WRAIR on August 9, 1999 (Lot No. 1N2-29-2).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	46.51	5.46	10.85
Found	46.52, 46.60	5.52, 5.60	10.86, 10.82

## Spectral Data

### FT-Infrared (KBr pellet)

$\nu$  3355, 3306, 3190, 1758, 1684, 1582, 1549,  
1463, 1376, 1345, 1253, 1214, 1189, 1167,  
1058, 1032, 997, 977, 882, 843, 823, 807,  
731, 704, 552, 578,  $441^{-1}$  cm.

### Nuclear Magnetic Resonance (400 MHz, $\text{CDCl}_3$ )

$^1\text{H}$   $\delta$  7.38 (s, 1, NH); 2.83 (d, 1,  $J=3.7$  Hz,  
CH); 0.91 (t, 2,  $\text{CH}_2$ ); 0.68 (t, 2,  $\text{CH}_2$ ).

$^{13}\text{C}$   $\delta$  161.3, 158.8, 22.5, 6.0.

## Thin Layer Chromatography

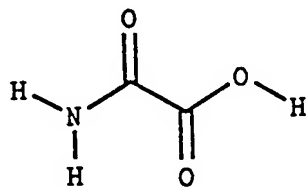
EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	$\text{CH}_3\text{OH}-\text{NH}_4\text{OH}-\text{CHCl}_3$ (1:1:6)	0.69
2.	$n\text{-BuOH}-\text{AcOH}-\text{H}_2\text{O}$ (5:1:2)	0.27

Source of Materials

1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. Toluene	Aldrich Chemical Co., Inc.
3. Pyridine	Aldrich Chemical Co., Inc.
4. Cyclopropylamine	Aldrich Chemical Co., Inc.
5. CH <sub>3</sub> OH	J.T. Baker Chemical Co.
6. EtOAc	J.T. Baker Chemical Co.
7. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.

9. Acetic acid, amino- (1)



1

The target compound 1 was purchased from Aldrich Chemical Company, Inc., Catalog No. 0-920-4, Lot No. JU 02708MS and characterized as 1N2-35-1, mp 206°C (d), literature<sup>8</sup> mp 210°C(d). A portion (2 g) was transmitted to WRAIR on September 23, 1999 (Lot No. 1N2-35-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>2</sub> H <sub>3</sub> NO <sub>3</sub>	26.98	3.40	15.73
Found	26.99	3.39	15.78

Spectral Data

FT-Infrared (KBr pellet)

ν 3356, 3244, 2765, 2538, 2457, 1882, 1736, 1678, 1471, 1362, 1238, 1085, 979, 835, 813, 754, 685, 661, 552, 477, 454 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 13.41 (broad s, 1, OH); 8.07 (s, 1, NH);  
7.75 (s, 1H).

<sup>13</sup>C δ 162.8, 160.7.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

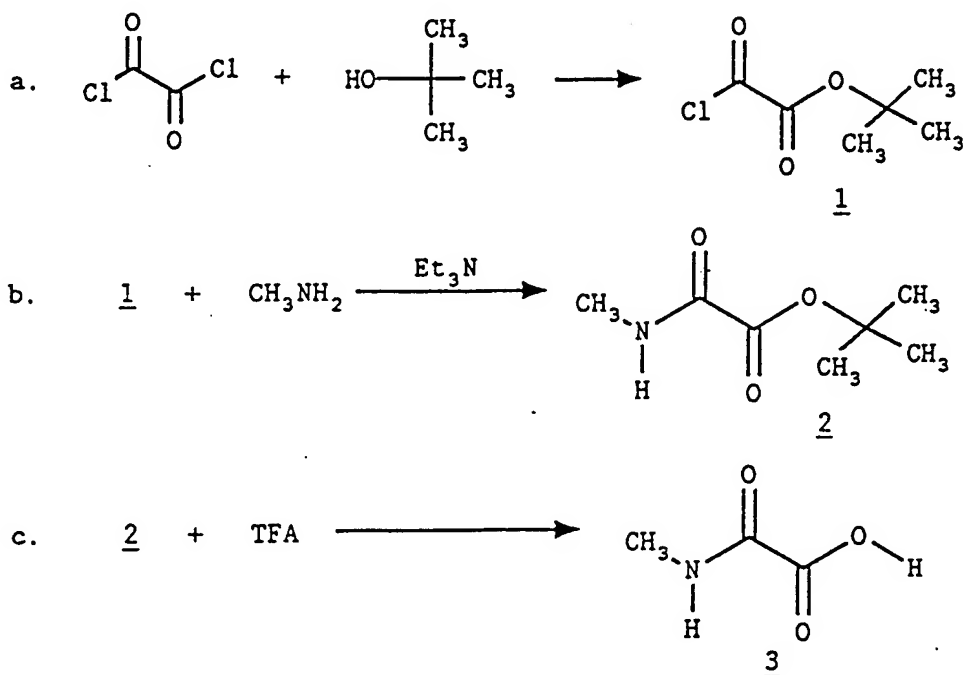
	<u>Eluent</u>	<u>Rf</u>
1.	CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.71
2.	n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.06



10. Acetic acid, (methylamino)oxo- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence



Experimental<sup>7</sup>

tert-Butyl chloroglyoxalate (1)

To cold (-8°C) oxalyl chloride (25.8 g, 17.7 mL, 0.203 mol) was added, dropwise tert-butyl alcohol (7.5 g, 9.7 mL, 0.101 mol). A mild exotherm was observed. The addition required 54 min. After the addition the reaction mixture was allowed to warm to room temperature. During the warm-up period effervescence was observed which increased proportionately with temperature. The mixture was stirred overnight. Excess oxalyl chloride was removed in vacuo with significant foaming. The residual liquid (9.8 g) was

transferred to another flask then distilled. The fraction, bp 56°/21-23 mm Hg was collected; literature<sup>1</sup> bp 60-63°/23 mm Hg; yield 9.4 g (56.5%). The material was suitable for further transformation.

Acetic acid, (methylamino)oxo-, tert-butyl ester (2)

To cooled (1°C), stirred CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added a 2M solution of methylamine in THF (70 mL, 14 mmol). A solution of 1 (2.30 g, 14 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added, dropwise, over 20 min. A solution of triethylamine (1.96 mL, 1.42 g, 14.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added, dropwise, over 15-20 min. The mixture was stirred 2 h in an ice bath then allowed to stand over the weekend. The mixture was transferred to a separatory funnel with the aid of CH<sub>2</sub>Cl<sub>2</sub> (5 mL) then washed with 1N HCl (3 x 15 mL). The combined acid washes were extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL). Organic portions were combined, dried (MgSO<sub>4</sub>), then concentrated to an oil (2.10 g). The oil was dissolved in hexane:EtOAc (1:1) (10 mL), applied to a 110 g column (4 x 18.5 cm) of silica gel, and eluted with hexane:EtOAc (1:1). The following fractions (60 mL each) were collected. Fractions 1-2 were recycled, fractions 3-8 were discarded, fractions 9-15 containing product were concentrated to 1.52 g of colorless oil. The material was suitable for further transformation.

Acetic acid, (methylamino)oxo- 3

To a stirred, cooled (4°C) solution of 2 (1.52 g, 9.55 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7.5 mL) was added CF<sub>3</sub>CO<sub>2</sub>H (7.5 mL, 11.1 g, 97.4 mmol), dropwise, over 10 min. After the addition the cooling bath was removed, and the mixture was stirred for 15 min while warming to room temperature. The solvent was removed in vacuo to give 0.97 g of 3 as a white solid, mp

154.5-155.5°C. A portion (0.75 g) was transmitted to WRAIR on September 23, 1999 (Lot No. 1N2-45-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>3</sub> H <sub>5</sub> NO <sub>3</sub>	34.96	4.89	13.59
Found	35.15	4.97	13.45

Spectral Data

FT-Infrared (KBr pellet)

ν 3363, 3192, 1761, 1683, 1565, 1414, 1357, 1259, 1184, 1031, 865, 843, 809, 727, 540, 499, 411 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (400 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 13.71 (broad s, 1, CO<sub>2</sub>H); 8.73 (s, 1, NH); 2.64 (s, 3, CH<sub>3</sub>).  
<sup>13</sup>C δ 162.1, 158.7, 25.9.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254, 0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.75
2.	n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.14

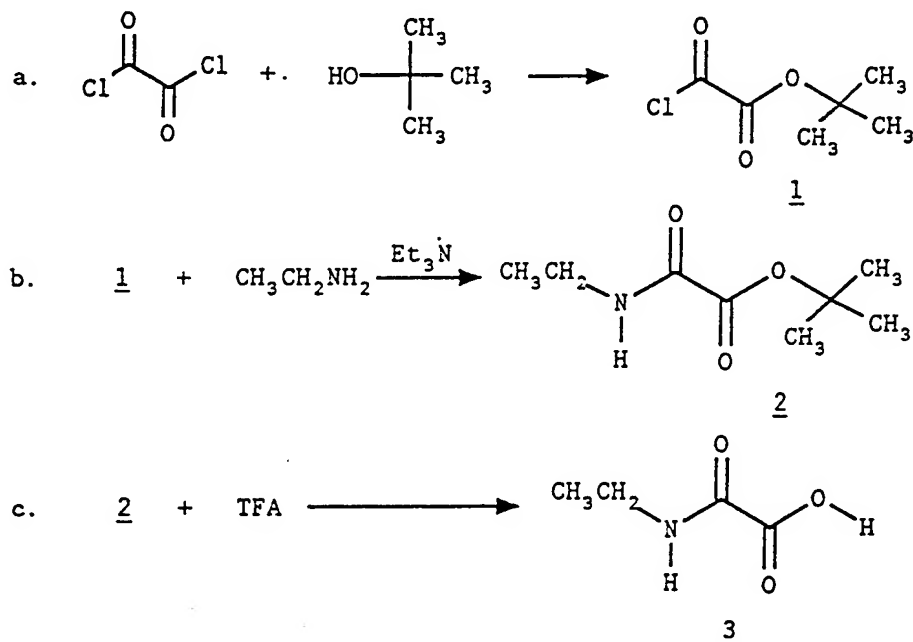
Source of Materials

1. Oxalyl chloride	Aldrich Chemical Co., Inc.
2. <i>tert</i> -Butyl alcohol	Aldrich Chemical Co., Inc.
3. <i>tert</i> -Butyl chloro- glyoxalate	Starks Associates, Inc.
4. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
5. Methylamine (2M in THF)	Aldrich Chemical Co., Inc.
6. Et <sub>3</sub> N	Aldrich Chemical Co., Inc.
7. HCl	J.T. Baker Chemical Co.
8. Hexane	J.T. Baker Chemical Co.
9. EtOAc	J.T. Baker Chemical Co.
10. Silica gel	Merck & Co, Inc.
11. Acetic acid, (methylamino)oxo-	Starks Associates, Inc.
12. Trifluoroacetic acid	Aldrich Chemical Co., Inc.

11. Acetic acid, (ethylamino)oxo- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

tert-Butyl chloroglyoxalate (1)

Please refer to page 45, this report.

Acetic acid, (ethylamino)oxo-, tert-butyl ester (2)

To cooled (1°C), stirred CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added a 2M solution of ethylamine in THF (60 mL, 12 mmol). The solution was cooled (1°C), and a solution of 1 (1.97 g,

12.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) was added, dropwise, over 20 min. A solution of triethylamine (1.67 mL, 1.21 g, 12.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) was added, dropwise, over 15 min, and the mixture was stirred in an ice bath for 2 h then allowed to stand over the weekend. The mixture was transferred to a separatory funnel with the aid of 5 mL of  $\text{CH}_2\text{Cl}_2$ , and washed with 1N HCl (3 x 15 mL). The combined acid washes were extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 15 mL). Organic portions were combined, dried ( $\text{MgSO}_4$ ), then concentrated to an oil (1.84 g). The oil was dissolved in 10 mL of hexane-EtOAc (1:1) then applied to a 110 g column (4 x 18.5 cm) of silica gel and eluted with hexane-EtOAc (1:1). The following fractions (60 mL each) were collected. Fraction 1-2 were recycled, fractions 3-5 were discarded, fractions 6-9 containing product + impurities were concentrated to give 1.15 g of clear oil. Additional product of similar purity (1.25 g) was obtained from 7 mL of the 2M solution of ethylamine in THF as starting material. The two lots were combined, dissolved in hexane-EtOAc (1:1) (12 mL) and rechromatographed on a 110 g of silica gel to give 1.90 g of pure 2; mp 56-57°C. The material was suitable for further transformation.

Acetic acid, (ethylamino)oxo- (3)<sup>7</sup>

To a stirred mixture of  $\text{CH}_2\text{Cl}_2$ -trifluoroacetic acid (1:1) (17 mL) at 4°C was added 2 (1.89 g, 10.9 mmol), and the mixture was stirred until the solid dissolved then the cooling bath was removed, and the mixture was stirred for 15 min while warming to room temperature. The solvent was removed in vacuo to give pure 3 (1.25 g, 97.6%), mp 132.5-134°C. A portion (1.0 g) was transmitted to WRAIR on September 23, 1999 (Lot No. 1N2-48-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_4H_7NO_3$	41.03	6.03	11.96
Found	41.05	6.11	11.93

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  3526, 3081, 2994, 2953, 2894, 1769, 1673,  
1555, 1476, 1392, 1370, 1338, 1256, 1181,  
1138, 1060, 914, 793, 716, 506, 411  $cm^{-1}$ .

Nuclear Magnetic Resonance (400 MHz, DMSO- $d_6$ )

$^1H$   $\delta$  13.70 (broad s, 1,  $CO_2H$ ); 8.80 (s, 1,  
NH); 3.10 (q, 2,  $J=6.9$  Hz,  $CH_2$ ); 1.00  
(t, 3,  $J=6.9$  Hz,  $CH_3$ ).

$^{13}C$   $\delta$  163.0, 158.7, 34.6, 15.1.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	$CH_3OH-NH_4OH-CHCl_3$ (1:1:6)	0.78
2.	$n-BuOH-AcOH-H_2O$ (5:1:2)	0.09

Source of Materials

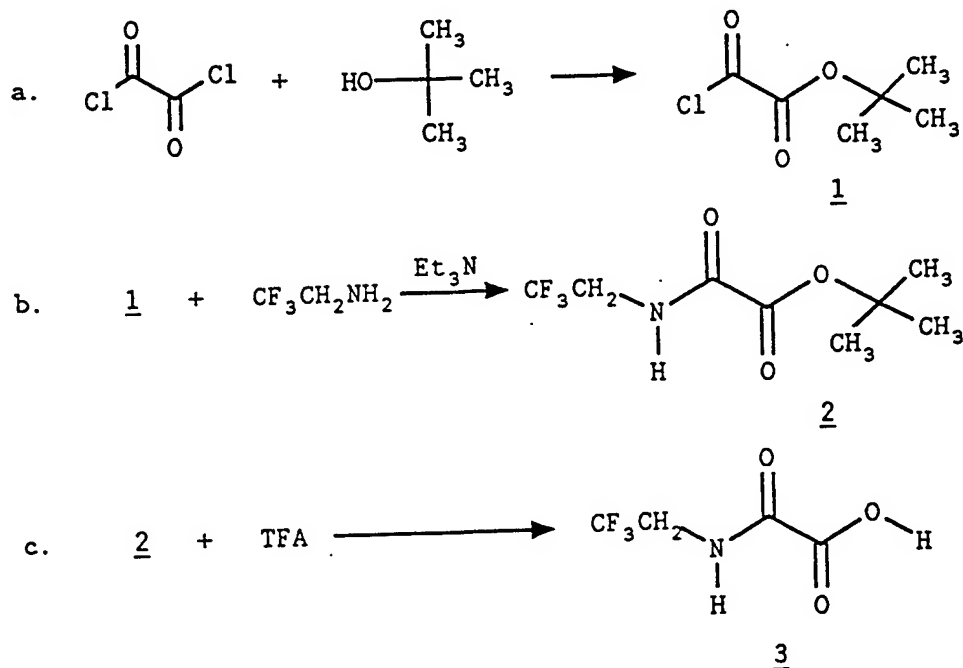
1. Oxalyl chloride	Aldrich Chemical Co., Inc.
2. <i>tert</i> -Butyl alcohol	Aldrich Chemical Co., Inc.
3. <i>tert</i> -Butyl chloro- glyoxalate	Starks Associates, Inc.
4. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
5. Ethylamine (2M in THF)	Aldrich Chemical Co., Inc.
6. Et <sub>3</sub> N	Aldrich Chemical Co., Inc.
7. HCl	J.T. Baker Chemical Co.
8. Hexane	J.T. Baker Chemical Co.
9. EtOAc	J.T. Baker Chemical Co.
10. Silica gel	Merck & Co, Inc.
11. Acetic acid, (ethylamino)oxo-, <i>tert</i> -butyl ester	Starks Associates, Inc.
12. Trifluoroacetic acid	Aldrich Chemical Co., Inc.



12. Acetic acid, oxo[(2,2,2-trifluoroethyl)amino] - (3)

The target compound 3 was prepared by the following sequence of reactions..

Reaction Sequence:



Experimental

tert-Butyl chloroglyoxalate (1)

Please refer to page 45, this report.

Acetic acid, oxo[(2,2,2-trifluoroethyl)amino]-, tert-butyl ester (2)

A stirred mixture of 2,2,2-trifluoroethylamine, hydrochloride (4.10 g, 30.3 mmol) and triethylamine (3.06 g, 4.22 mL, 30.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was cooled to -7°C.. A solution of acid chloride 1 (5.00 g, 30.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added, dropwise, over 15 min (exotherm to 1°C).

The mixture was stirred for 4 min. A solution of Et<sub>3</sub>N (3.06 g, 4.22 mL, 30.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added, dropwise over 6 min, and the mixture was stirred for 2 h at -4 to -1°C, then allowed to warm to room temperature. The mixture was washed with 1N HCl (3 x 25 mL), and the combined washes were extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 25 mL). The organic portions were combined, dried (MgSO<sub>4</sub>), then concentrated in vacuo to a white solid (6.45 g). The solid was dissolved in EtOAc-hexane (1:1) and applied to a 110 g column of silica gel and eluted with hexane-EtOAc (1:1). Fractions (60 mL each) 4-6 were combined then concentrated in vacuo to yield 6.18 g of 2 with some impurities. The material was rechromatographed on the same size column to give 5.51 g of pure 2, mp 86.5 - 87°C. The material was suitable for further transformation.

Acetic acid, oxo[(2,2,2-trifluoroethyl)amino] - (3)

To a stirred, cooled (3°C), solution of trifluoroacetic acid (7 mL) in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) was added 2 (1.95 g, 8.58 mmol) in one portion, and the mixture was stirred until a solution was obtained. The cooling bath was removed, and the solution was allowed to warm to room temperature (~15 min) then concentrated in vacuo to a white solid (1.46 g). The solid was dissolved in EtOAc (10 mL), and the solution was diluted with hexane (30 mL). The solution was concentrated to ~10 mL, and the solid that separated was collected then dried, yield 1.265 g, mp 132-133°C. A portion (1.06 g) was transmitted to WRAIR on September 23, 1999 (Lot No. 1N2-59-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_4H_4F_3NO_3$	28.08	2.36	8.19
Found	28.24	2.30	8.15

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  3352, 3300, 3115, 3030, 2986, 2927,  
2855, 2805, 1766, 1703, 1692, 1569,  
1434, 1399, 1365, 1296, 1274, 1251,  
1188, 1156, 1079, 986, 864, 838, 770,  
720, 671, 573, 539, 503, 485, 458  $cm^{-1}$ .

Nuclear Magnetic Resonance (200 MHz, DMSO- $d_6$ )

$^1H$   $\delta$  14.1 (broad s, 1,  $CO_2H$ ); 9.38 (t, 1,  
J=6.2 Hz, NH); 3.92 (m, 2,  $CH_2$ ).  
 $^{13}C$   $\delta$  157.1, 155.0, 122.0, 118.3.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	$CH_3OH-NH_4OH-CHCl_3$ (1:1:6)	0.84
2.	$n-BuOH-AcOH-H_2O$ (5:1:2)	0.45

Source of Materials

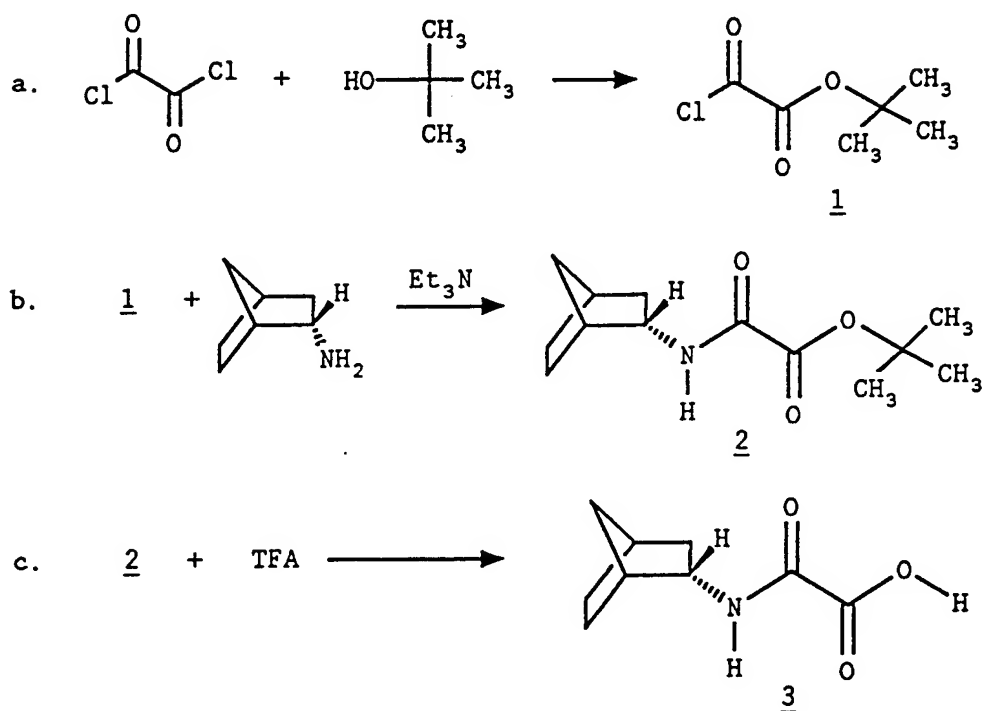
1. Oxalyl chloride	Aldrich Chemical Co., Inc.
2. tert-Butyl alcohol	Aldrich Chemical Co., Inc.
3. tert-Butyl chloro- glyoxalate	Starks Assoc., Inc.
4. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
5. (2,2,2-Trifluoroethyl)- amine, hydrochloride	Aldrich Chemical Co., Inc.
6. Et <sub>3</sub> N	Aldrich Chemical Co., Inc.
7. HCl	J.T. Baker Chemical Co.
8. EtOAc	J.T. Baker Chemical Co.
9. Hexane	J.T. Baker Chemical Co.
10. Silica gel	Merck & Co, Inc.
11. Acetic acid, oxo[(2,2,2- trifluoroethyl)amino)-, tert-butyl ester	Starks Associates, Inc.
12. Trifluoroacetic acid	Aldrich Chemical Co., Inc.

13. Acetic acid, [(bicyclo[2.2.1]hept-2-yl)amino]oxo-,  
endo- (3)

---

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

tert-Butyl chloroglyoxalate (1)

Please refer to page 45, this report.

Acetic acid, [(bicyclo[2.2.1]hept-2-yl)amino]oxo-,  
endo-, tert-butyl ester (2)

---

To a cooled (0°C), stirred solution of [bicyclo[2.2.1]hept-2-yl]amine hydrochloride, endo- (1.48 g, 10.0 mmol) and Et<sub>3</sub>N (2.02 g, 20.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (22.5 mL) was added a solution of 1 (1.65 g, 10.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) dropwise, and the mixture was stirred for 2 h. The solid that separated was collected on a filter, and the filtrate was washed with 1N HCl (3 x 10 mL), dried (MgSO<sub>4</sub>), then concentrated in vacuo to a solid. The solid was purified by chromatography on a column of silica gel (100 g) using hexane-EtOAc (3:2) as the eluent. Fractions containing product were combined then concentrated in vacuo, yield, 1.719 g. The material was suitable for further transformation.

Acetic acid, [(bicyclo[2.2.1]hept-2-yl)amino]oxo-,  
endo- (3)

---

To a cooled (0°C) solution of 2 (1.710 g, 6.866 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added trifluoroacetic acid (6 mL) in 1 mL portions. After the addition the mixture was stirred at RT for 17 h then concentrated in vacuo to a solid residue. The solid was crystallized from hexane-EtOAc (6:4) (~200 mL) to give pure 3, mp 165-166°C.<sup>9</sup> A portion (0.88 g) was transmitted to WRAIR on September 23, 1999 (Lot No. 2N2-06-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>9</sub> H <sub>13</sub> NO <sub>3</sub>	59.00	7.15	7.65
Found	59.11	7.15	7.61

## Spectral Data

### FT-Infrared (KBr pellet)

$\nu$  3332, 3174, 2956, 2872, 1763, 1684,  
1554, 1476, 1451, 1381, 1351, 1310,  
1263, 1250, 1186, 1160, 1122, 1098,  
1046, 1004, 921, 897, 843, 801, 720,  
594, 522, 478  $\text{cm}^{-1}$ .

### Nuclear Magnetic Resonance (200 MHz, DMSO- $d_6$ )

$^1\text{H}$   $\delta$  7.36 (broad s, 1, NH); 4.10 (m, 1, CHN); 2.49 (m, 1); 2.28 (m, 1); 2.12 (m, 1); 1.61 (m, 1); 1.45 (m, 3); 1.40 (m, 1); 1.28 (m, 1); 0.91 (m, 1).

$^{13}\text{C}$   $\delta$  155.5, 152.9, 47.7, 35.8, 33.8, 32.7, 32.1, 25.2, 17.1.

## Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254, 0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>R<sub>f</sub></u>
1. $\text{CH}_3\text{OH}-\text{NH}_4\text{OH}-\text{CHCl}_3$ (1:1:6)	0.83
2. $n\text{-BuOH}-\text{AcOH}-\text{H}_2\text{O}$ (5:1:2)	0.58

Source of Materials

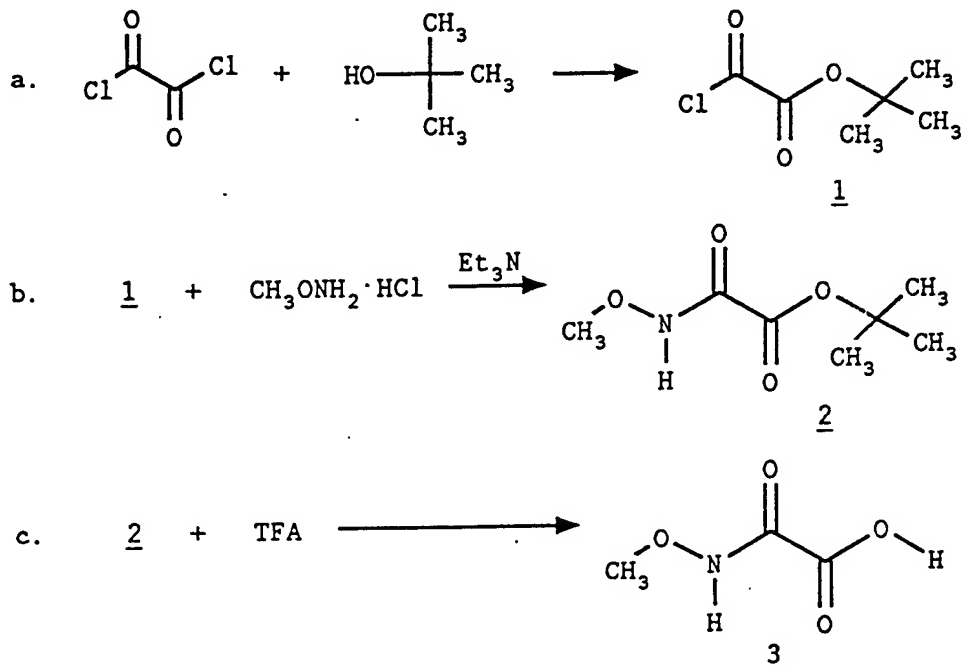
- |  |                            |
|--|----------------------------|
| 1. Oxalyl chloride   | Aldrich Chemical Co., Inc. |
| 2. <i>tert</i> -Butyl alcohol  | Aldrich Chemical Co., Inc. |
| 3. <i>tert</i> -Butyl chloro-<br>glyoxalate  | Starks Associates, Inc.    |
| 4. CH <sub>2</sub> Cl <sub>2</sub>   | J.T. Baker Chemical Co.    |
| 5. [Bicyclo[2.2.1]hept-2-yl]amine,<br>hydrochloride, <i>endo</i> -                                     | Aldrich Chemical Co., Inc. |
| 6. Et <sub>3</sub> N   | Aldrich Chemical Co., Inc. |
| 7. HCl   | J.T. Baker Chemical Co.    |
| 8. Hexane  | J.T. Baker Chemical Co.    |
| 9. EtOAc   | J.T. Baker Chemical Co.    |
| 10. Silica gel   | Merck & Co, Inc.           |
| 11. Acetic acid, [(bicyclo[2.2.1]-<br>hept-2-yl)amino]oxo-, <i>endo</i> -,<br><i>tert</i> -butyl ester | Starks Associates, Inc.    |
| 12. Trifluoroacetic acid   | Aldrich Chemical Co., Inc. |



14. Acetic acid, (methoxyamino)oxo- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

tert-Butyl chloroglyoxalate (1)

Please refer to page 45, this report.

Acetic acid, (methoxyamino)oxo-, tert-butyl ester (2)

To a cooled (1°C), stirred solution of methoxylamine, hydrochloride (1.00 g, 12.0 mmol) and triethylamine (1.21 g, 1.67 mL, 12.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added, dropwise, a solution of 1 (1.97 g, 12.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) over 20 min. The stirring was continued until the temperature of the mixture reached 1°C. A solution of triethylamine (1.21 g, 1.67 mL, 12.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added, dropwise, over 20 min, and the mixture was stirred for 2 h in an ice bath then allowed to warm to room temperature. The solution was washed with 1N HCl (3 x 10 mL), and the combined acid washes were extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The organic portions were combined, dried (MgSO<sub>4</sub>), then concentrated to an oil (3.07 g, >100%). The material was purified by chromatography on a column of SiO<sub>2</sub> (110 g) using hexane-EtOAc (1:1) as the eluent. Fractions (60 mL each) 8-17 contained pure 2. These were combined then concentrated in vacuo to give 1.8 g (85.7%) of pure 2. The material was suitable for further transformation. Additional product (1.38 g) was obtained from an identical reaction.

Acetic acid, (methoxyamino)oxo- (3)

To a cooled (4°C), stirred solution of 2 (1.80 g, 10.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (9 mL) was added trifluoroacetic acid (13.3 g, 9 mL, 117 mmol) in five portions. The first portion (2 mL) resulted in an exotherm, to 10°C. After the addition the cooling bath was removed, and the mixture was stirred at room temperature for ½ h. The solvents were removed in

vacuo leaving a residue of white solid (1.2 g), mp 138-139°C; literature<sup>5</sup> mp 132-135°C. The material was recrystallized from hexane-EtOAc (2:1) to give 785 mg of 3. Additional 3 (734 mg) of similar purity was obtained from another reaction. The lots were combined then recrystallized from EtOAc/hexane (1:1) (40 mL each); yield 1.178 g (77.6% recovery) mp 136-137°C(d); literature<sup>5</sup> mp 132-135°C. A portion (0.988 g) was transmitted to WRAIR on October 27, 1999 (Lot No. 1N2-58-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>3</sub> H <sub>5</sub> NO <sub>4</sub>	30.26	4.23	11.76
Found	30.46, 30.54	4.22, 4.27	11.58, 11.66

Spectral Data

FT-Infrared (KBr pellet)

ν 3282, 2781, 2550, 2488, 1715, 1683,  
1660, 1528, 1457, 1430, 1418, 1287,  
1233, 1151, 1067, 960, 901, 818, 745,  
724, 662, 554, 518, 464 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (500 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 14.12 (broad s, 1, CO<sub>2</sub>H); 12.16 (s,  
1, NH); 3.63 (s, 3, CH<sub>3</sub>).  
<sup>13</sup>C δ 161.8, 155.6, 63.9.

### Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>R<sub>f</sub></u>
1. CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.51
2. n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.07

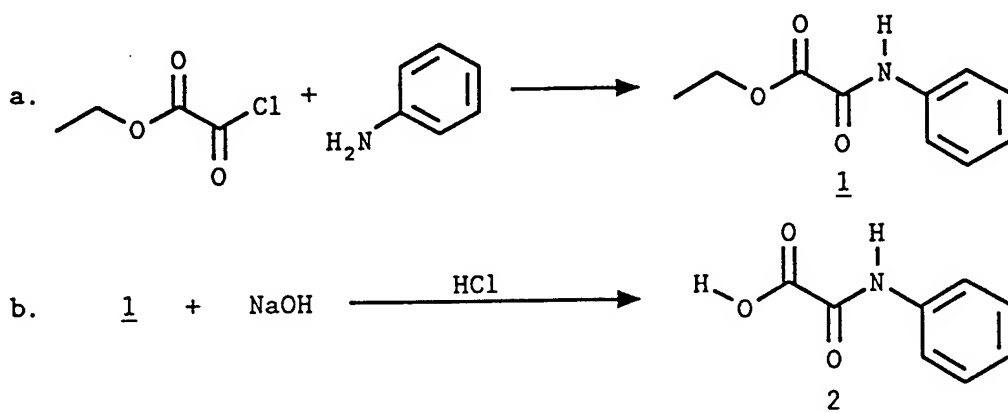
### Source of Materials

1. Oxalyl chloride	Aldrich Chemical Co., Inc.
2. tert-Butyl alcohol	Aldrich Chemical Co., Inc.
3. tert-Butyl chloro- glyoxalate	Starks Associates, Inc.
4. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
5. Methoxylamine, HCl	Aldrich Chemical Co., Inc.
6. Et <sub>3</sub> N	Aldrich Chemical Co., Inc.
7. HCl	J.T. Baker Chemical Co.
8. Hexane	J.T. Baker Chemical Co.
9. EtOAc	J.T. Baker Chemical Co.
10. Silica gel	Merck & Co, Inc.
11. Acetic acid, (methoxyamino)oxo-, tert-butyl ester	Starks Associates, Inc.
12. Trifluoroacetic acid	J.T. Baker Chemical Co., Inc.

15. Acetic acid, oxo(phenylamino)- (2)

The target compound 2 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Acetic acid, (oxo(phenylamino)-, ethyl ester (1)

To a cold (0°C), stirred solution of ethyl oxalyl chloride (14.6 g, 0.107 mol) and pyridine (12.0 g, 0.150 mol) in toluene (50 mL) was added aniline (10.0 g, 0.107 mol), dropwise, while maintaining the reaction temperature at 0°C. The stirred suspension was allowed to warm to room temperature then stirred for 1 h. Methanol (25 mL) was slowly added to the reaction mixture, and the resulting solution was concentrated in vacuo to a solid residue. The residue was extracted with EtOAc (3 x 50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated in vacuo to an oil (21.0 g). The

oil was purified by chromatography on a column of SiO<sub>2</sub> (150 g) using hexane-EtOAc (1:1) as the eluent. Fractions containing pure 1 were concentrated in vacuo to give 17.4 g (84.1%) of pure 1. The material was suitable for further transformation.

Acetic acid, oxo(phenylamino)- (2)<sup>10</sup>

To a stirred solution of 1 (10.0 g, 0.0517 mol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added, dropwise, a solution of NaOH (2.1 g, 0.0525 mol) in H<sub>2</sub>O (50 mL). The solution became solid after ~2 min of stirring. MeOH (200 mL) was added, and the suspension was stirred for 2 h. The mixture was concentrated in vacuo to remove MeOH, and the slurry was diluted with H<sub>2</sub>O (250 mL). To the suspension was slowly added a solution of conc. HCl (5.2 mL) in H<sub>2</sub>O (20 mL), and the suspension was stirred for 2 h. The solid was collected on a filter, washed with H<sub>2</sub>O (50 mL), then dried (MgSO<sub>4</sub>), yield 6.3 g (73.8%). The material was crystallized from AcOH (150 mL) to give 3.7 g of 2. Additional product of comparable purity (2.8 g) was obtained from a smaller reaction starting with 7.4 g of 1. The 2.8 g lot was dissolved in 1.75M NaOH (300 mL). The solution was filtered then added, dropwise, to 1 L of 0.6M HCl. The acidic solution was cooled then extracted with ether (4 x 400 mL). The extracts were combined, dried (MgSO<sub>4</sub>), then concentrated in vacuo to a solid (1.187 g). The solid was triturated with hexane (10 mL), collected, washed with hexane (10 mL), then dried (MgSO<sub>4</sub>) to yield 1.056 g; mp 152.5-153.5°C, literature<sup>11</sup> mp 150°C. A portion (0.856 g) was transmitted to WRAIR on October 27, 1999 (Lot No. 1N2-60-2).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_8H_7NO_3$	58.19	4.27	8.48
Found	57.92	4.28	8.45

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  3306, 1767, 1685, 1603, 1549, 1497,  
1450, 1351, 1313, 1247, 1211, 1178,  
1167, 1080, 1028, 938, 900, 755, 732,  
691, 539, 518, 468  $cm^{-1}$ .

Nuclear Magnetic Resonance (500 MHz, DMSO- $d_6$ )

$^1H$   $\delta$  14.19 (broad s, 1,  $CO_2H$ ); 10.71 (s,  
1, NH); 7.77 (d, 2,  $J=7.9$  Hz, H's at C-  
3 and C-5); 7.36 (t, 2,  $J=7.6$  Hz, H's  
at C-2 and C-6); 7.14 (t, 1,  $J=7.3$  Hz,  
H at C-4).

$^{13}C$   $\delta$  162.9, 157.5, 138.3, 129.4, 125.2,  
121.0.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	$CH_3OH-NH_4OH-CHCl_3$ (1:1:6)	0.68
2.	$n-BuOH-AcOH-H_2O$ (5:1:2)	0.43

Source of Materials

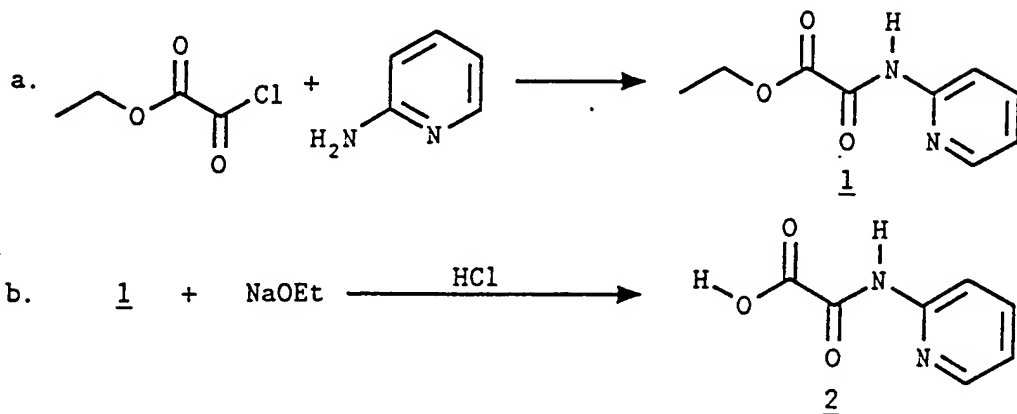
1. Ethyl oxalyl chloride	Aldrich Chemical Co., Inc.
2. Pyridine	Aldrich Chemical Co., Inc.
3. Aniline	Lancaster Synthesis
4. Methanol	J.T. Baker Chemical Co.
5. Toluene	Aldrich Chemical Co., Inc.
6. Ethyl acetate	J.T. Baker Chemical Co.
7. Silica gel	Merck & Co, Inc.
8. Hexane	J.T. Baker Chemical Co.
9. NaOH	J.T. Baker Chemical Co.
10. HCl	J.T. Baker Chemical Co.
11. Ether	Fisher Scientific



16. Acetic acid, oxo(2-pyridinylamino)- (2)

The target compound 2 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Acetic acid, oxo(2-pyridinylamino)-, ethyl ester (1)

To a stirred, cooled (10-20°C) solution of 2-aminopyridine (36.5 g, 0.388 mol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) was added a solution of ethyl oxalyl chloride (27.3 g, 97%, 22.3 mL, 0.194 mol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL), dropwise, over a period of 30 min. The mixture was stirred at 10°C for 1 h, and the black suspension was washed with H<sub>2</sub>O (4 x 200 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) then concentrated in vacuo to a black residue (26.4 g). The residue was purified by chromatography on a column of silica gel using CH<sub>2</sub>Cl<sub>2</sub> then CH<sub>2</sub>Cl<sub>2</sub>-ether (19:1) as the eluent. Fractions containing pure product were combined, then concentrated in vacuo to give 16.6 g (44.1%) of 1 as colorless oil. The material was suitable for further transformation.

Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H δ 9.54 (broad s, 1, NH); 8.36 (dd, 1, J=4.8 Hz, 1.1 Hz, H at C-6); 8.25 (d, 1, J=8.4 Hz, H at C-3); 7.77 (d of t, 1, J=7.9 Hz, 1.8 Hz, H at C-4); 7.13 (m, 1, H at C-5); 4.43 (q, 2, J=7.0 Hz, CH<sub>2</sub>); 1.43 (t, 3, J=7.3 Hz, CH<sub>3</sub>).

Acetic acid, oxo(2-pyridinylamino)- (2)

Sodium ethoxide solution was prepared by heating 1.86 g (80.8 mmol) of Na in 100 mL of ethanol. When all the sodium had dissolved and the evolution of hydrogen had ceased a solution of 1 (15.7 g, 80.8 mmol) in ethanol (70 mL) was added dropwise to the hot sodium ethoxide solution. A white precipitate formed almost immediately. The mixture was heated under reflux for 1 h. TLC showed presence of starting material. The solid was collected on a filter, washed with ethanol, then dried to yield 4.8 g. The solid was dissolved in H<sub>2</sub>O (50 mL), the solution was filtered, and the filtrate was stirred in a solution of conc. HCl/H<sub>2</sub>O (4 mL/50 mL) for 15 min. The precipitate was collected then dried, yield 1.2 g, mp ~216°C. Two additional lots of sodium salt separated from the filtrate. These were acidified in the same way to give 1.3 g, mp ~225°C and 0.8 g, mp ~224°C of 2. The three lots were combined then recrystallized from H<sub>2</sub>O (200 mL); yield 2.5 g (71.4% recovery), mp 227-228°C; literature<sup>12,13</sup> mp 197-198°C for the anhydrous form and 231-232°C for mono-hydrate, respectively. A portion (2.3 g) was transmitted to WRAIR on October 27, 1999 (Lot No. 1N2-65-3).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_7H_6N_2O_3$	45.31	3.22	13.55
· 0.3H <sub>2</sub> O · 0.8CO <sub>2</sub>			
Found	45.41, 45.47	3.40, 3.41	13.18, 13.23

Spectral Data

FT-Infrared (KBr pellet)

ν 3089, 1920, 1740, 1718, 1698, 1642, 1606,  
1558, 1499, 1439, 1391, 1338, 1252, 1217,  
1191, 1160, 1093, 1036, 922, 864, 782,  
705, 601, 528, 484, 442 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (400 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 13.63 (broad s, 1.8 H, CO<sub>2</sub>H); 10.47  
(broad s, 1, NH); 8.34 (d, 1, J=2.9 Hz,  
H at C-3); 7.98 (d, 1, J= 8.1 Hz, H at  
C-6); 7.82 (t, 1, J=7.7 Hz, H at C-5);  
7.16 (t, 1, J=5.5 Hz, H at C-4).

<sup>13</sup>C δ 162.5, 161.6, 158.4, 150.9, 148.9,  
139.3, 121.3, 114.9.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

	<u>Eluent</u>	<u>Rf</u>
1.	CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.66
2.	n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.20

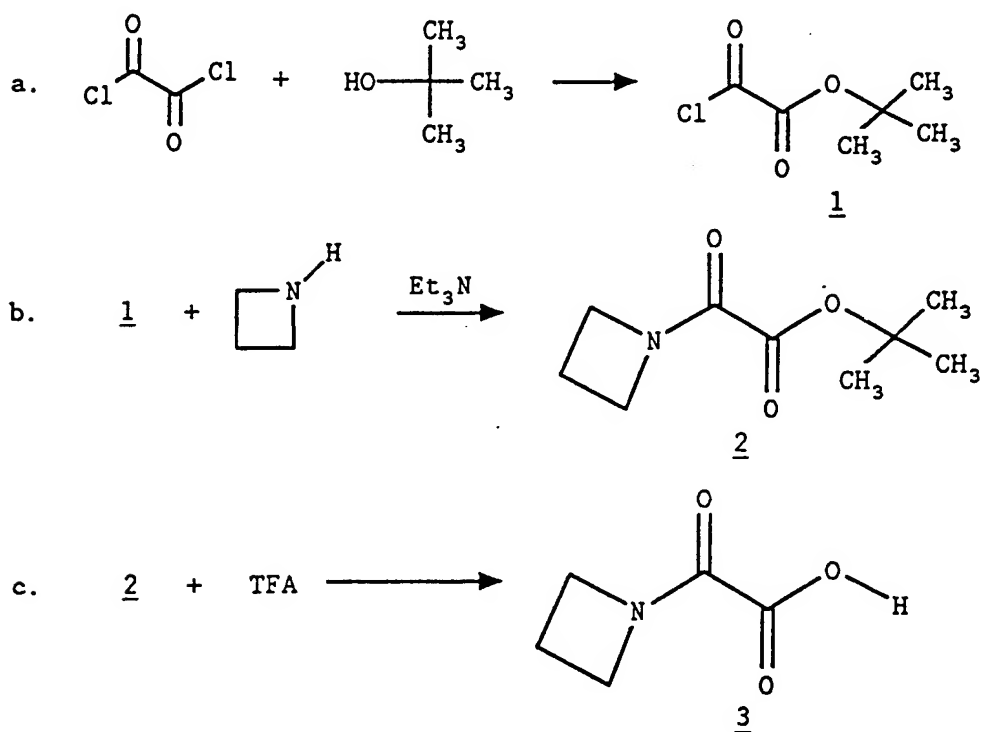
Source of Materials

- |   |                            |
|---|----------------------------|
| 1. 2-Aminopyridine  | Aldrich Chemical Co., Inc. |
| 2. CH <sub>2</sub> Cl <sub>2</sub>                          | J.T. Baker Chemical Co.    |
| 3. Ethyl oxalyl chloride                                    | Aldrich Chemical Co., Inc. |
| 4. Silica gel   | Merck & Co., Inc.          |
| 5. Ether  | Fisher Scientific          |
| 6. Acetic acid, oxo-<br>(2-pyridinylamino)-,<br>ethyl ester | Starks Assoc., Inc.        |
| 7. Sodium   | Aldrich Chemical Co., Inc. |
| 8. EtOH   | Aaper Alcohol & Chem. Co.  |
| 9. HCl  | J.T. Baker Chemical Co.    |

17. 1-Azetidineacetic acid, .alpha.-oxo- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

tert-Butyl chloroglyoxalate (1)

Please refer to page 45, this report.

1-Azetidineacetic acid, .alpha.-oxo-, tert-butyl ester (2)

To a cooled (0°C), stirred mixture of azetidine hydrochloride (1.08 g, 11.5 mmol), triethylamine (1.16 g, 1.60 mol, 11.5 mmol), and CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added, dropwise, over a period of 16 min a solution of 1 (1.90 g, 11.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) (exotherm to +8°C) followed by dropwise addition of a solution of triethylamine (1.16 g, 1.60 mL, 11.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL). The mixture was stirred at 0°C for 2 h then allowed to warm to RT. The solid was filtered off, and the filtrate was washed with 1N HCl (2 x 10 mL). The acid wash was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 10 mL). The combined organic portions were dried (MgSO<sub>4</sub>) then concentrated in vacuo to an oil (2.38 g). The oil was chromatographed on a column of SiO<sub>2</sub> (110 g) using hexane-EtOAc (1:1) as the eluent. Fractions 12-25 (50 mL each) containing pure product were concentrated in vacuo to give 1.733 g of pure (2). The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H δ 4.49 (t, 2, J=8.1 Hz, one H at C-2 and one H at C-4); 4.10 (t, 2, J=8.1 Hz, one H at C-2 and one H at C-4); 2.31 (t, 2, J= 7.7 Hz, 2H's at C-3); 1.51 (s, 9, tert-butyl).

1-Azetineacetic acid, .alpha.-oxo- (3)

To a cooled (1°C), stirred solution of 2 (1.72 g, 9.29 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8.2 mL) was added, dropwise, over 6 min, trifluoroacetic acid (8.2 mL). The solution was allowed to

warm to RT while stirring (~1/2 h). The solvent was removed in vacuo to give an oil which crystallized on standing. The material was dried in vacuo to give 1.118 g of a solid. The solid was heated in EtOAc (13 mL). A small amount of insolubles were filtered off, and the filtrate was diluted with hexane, dropwise, until the precipitation had stopped. The mixture was cooled, and the solid was collected. Concentration of the filtrate to a smaller volume yielded a second crop of 3. The two lots were combined then dried in vacuo at 40-45°C to give 0.84 g of 3, mp 110-114°C.<sup>9</sup> A portion (0.70 g) was transmitted to WRAIR on October 27, 1999 (Lot No. 1N2-66-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	46.52	5.46	10.85
Found	46.29	5.51	10.77

Spectral Data

FT-Infrared (KBr pellet)

ν 3447, 2964, 2893, 2560, 2480, 1923, 1731, 1634, 1503, 1452, 1430, 1298, 1248, 1198, 1147, 1120, 1018, 962, 932, 851, 815, 736, 672, 530, 437 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (500 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 13.75 (broad s, 1H, CO<sub>2</sub>H); 4.32 (t, 2H, J=7.3 Hz, 8.1 Hz, one H at C-2 and one H at C-4); 3.91 (t, 2H, J=8.3 Hz, 7.7 Hz, one H at C-2 and one H at C-4); 2.20 (q, 2H, J= 7.7 Hz, 2 H's at C-3).

<sup>13</sup>C δ 157.3, 154.1, 48.1, 43.8, 11.3.

Mass Spectroscopy (Electrospray ES<sup>-</sup>)

m/z = 128 [M-1]

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

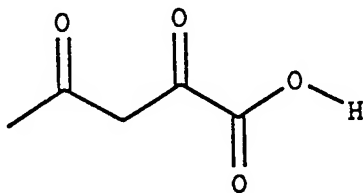
<u>Eluent</u>	<u>R<sub>f</sub></u>
1. CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.60
2. n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.09

Source of Materials

1. tert-Butyl chloroglyoxalate	Starks Associates, Inc.
2. Azetidine, HCl	Aldrich Chemical Co., Inc.
3. Triethylamine	Aldrich Chemical Co., Inc.
4. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
5. HCl	J.T. Baker Chemical Co.,
6. SiO <sub>2</sub>	Merck & Co., Inc.
7. EtOAc	J.T. Baker Chemical Co.
8. Hexane	J.T. Baker Chemical Co.
9. Azetidineacetic acid, .alpha.-oxo-, tert-butyl ester	Starks Associates, Inc.
10. TFA	Aldrich Chemical Co., Inc.



18. Pentanoic acid, 2,4-dioxo- (1)



1

The target compound (1) (700 mg) was purchased from Sigma Chemical Co. and characterized as 1N2-49-1. The material was not analytically pure and had to be purified. A portion (75 mg) was dissolved in ether (1 mL), and the solution was diluted with hexane (5 mL). The solution was concentrated to give an off-white solid. The solid was triturated with hexane to give 45 mg of 1. This material was used for characterization. The procedure was repeated with the remainder of starting material (575 mg); yield 353 mg, mp 97-97.5°C; literature<sup>14</sup> mp 55-63°C for monohydrate and 101°C for the anhydrous form. A portion (301 mg) was transmitted to WRAIR on October 27, 1999 (2N2-09-1).

Analysis:

	<u>C</u>	<u>H</u>
Calc'd for C <sub>5</sub> H <sub>6</sub> O <sub>4</sub>	46.16	4.62
Found	46.28	4.64

Spectral Data

FT-Infrared (KBr pellet)

ν 3214, 2577, 1762, 1738, 1601, 1384,  
1294, 1199, 1131, 994, 914, 836,  
801, 714, 564 cm<sup>-1</sup>.

Nuclear Magnetic Resonance (200 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 6.27 (s, 2, CH<sub>2</sub>); 3.96 (s, 1, CH=COH); 2.24 (s, 3, CH<sub>3</sub>); 2.18 (s, 3, CH<sub>3</sub>).

<sup>13</sup>C δ 203.1, 200.6, 165.1, 163.4, 161.2, 109.7, 102.5, 53.0, 30.4, 27.7.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254, 0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>R<sub>f</sub></u>
1. CH <sub>3</sub> OH-NH <sub>4</sub> OH-CHCl <sub>3</sub> (1:1:6)	0.59
2. n-BuOH-AcOH-H <sub>2</sub> O (5:1:2)	0.25

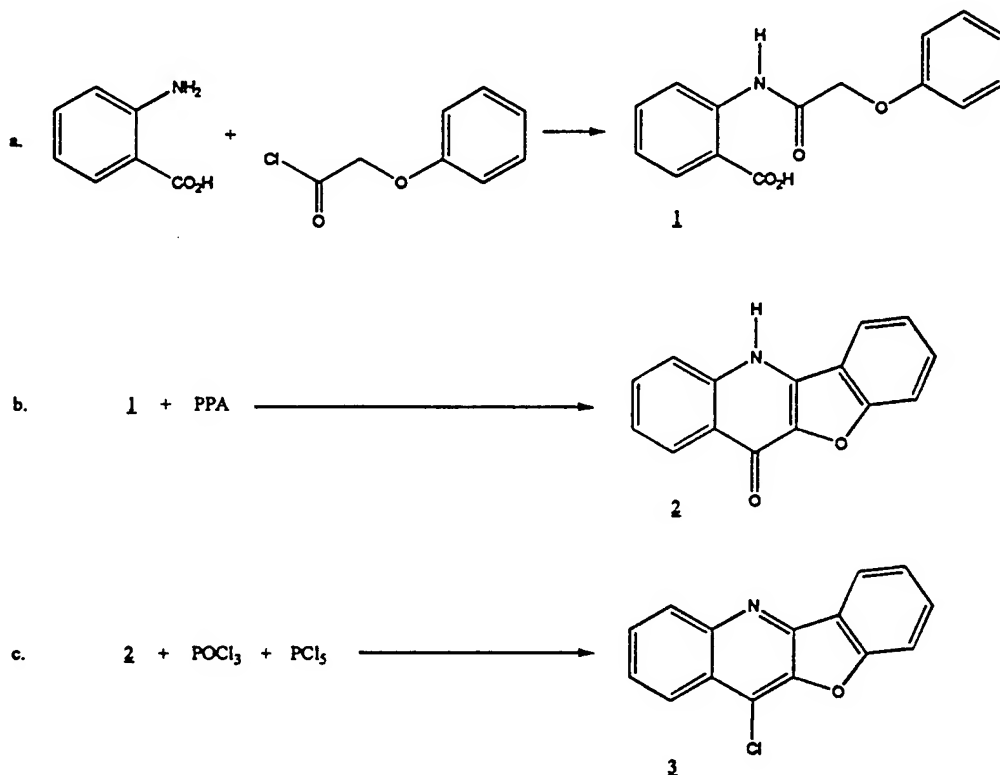
Source of Materials

1. Acetopyruvic acid	Sigma Chemical Co.
2. Ether	Fisher Scientific
3. Hexane	J.T. Baker Chemical Co.

19. Benzofuro[3,2-b]quinoline, 11-chloro- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Benzoic acid, 2-[[2-(phenoxy)acetyl]amino]- (1)

To a cooled (0°C), stirred solution of anthranilic acid (7.95 g, 57.9 mmol) in aq. NaOH (5 g, 60 mL) was added, dropwise (over 90 min), phenoxyacetyl chloride (9.88 g, 57.9 mmol). After the addition the mixture was stirred

at 0°C for 1 h then acidified with 1M HCl (60 mL). The solid was collected on a filter then taken up in EtOAc (125 mL). The insolubles were removed by filtration, and the filtrate was dried (MgSO<sub>4</sub>) then concentrated to a solid (11.61 g); mp 186-203°C. Additional product of similar purity (8.58 g) was obtained from another run. The filter cakes from both runs were combined, suspended in EtOAc (100 mL), and the suspension was stirred for 1 h then filtered. The filtrate was concentrated to 1.84 g of solid, mp 199-201°C. The filter cake was found to be the same material, mp 200-202°C. The filter cake was dried to give 7.62 g of product. Earlier lots of 8.58 g and 11.61 g were combined then stirred in 250 mL of hexane-EtOAc (1:1). The solid was collected then dried, yield 14.87 g, mp 200-201°C, total yield from both reactions, 24.4 g (72.9%). The material was suitable for further transformation.

### Spectral Data

#### Nuclear Magnetic Resonance (200 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 13.72 (s, 1H, CO<sub>2</sub>H); 12.16 (s, 1H, NH); 8.69 (d, 1H, J=8.4 Hz); 8.00 (dd, 1H, J=7.9 Hz, 1.0 Hz); 7.61 (t, 1H, J=8.3 Hz); 7.36-7.31 (m, 2H); 7.21-7.16 (m, 1H); 7.06-7.00 (m, 2H); 6.97 (m, 1H); 4.71 (s, 2H, CH<sub>2</sub>).

#### Benzofuro[3,2-b]quinolin-11(5H)-one (2)

A stirred mixture of 1 (5.0 g, 18.4 mmol) and PPA (25 g) was heated at 120-125°C for 1 h then poured over crushed ice (125 g) and neutralized with solid K<sub>2</sub>CO<sub>3</sub>. The resulting

precipitate was filtered off, washed with hot H<sub>2</sub>O (500 mL), and dried to give 4.0 g of product 2, mp >300°C. Additional product (10.5 g) was obtained from a larger reaction. The material was suitable for further transformation.

### Spectral Data

#### Nuclear Magnetic Resonance (400 MHz, DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 12.95 (broad s, 1H, NH); 8.35 (d, 1H, J=8.1 Hz); 8.24 (d, 1H, J=8.1 Hz); 7.83 (d, 1H, J=8.4 Hz); 7.76-7.63 (m, 3H); 7.51 (t, 1H, J=7.7 Hz); 7.18 (d, 1H, J=7.7 Hz).

#### Benzofuro[3,2-b]quinoline, 11-chloro- (3)

A stirred mixture of 2 (4.00 g, 17.0 mmol) and phosphorus pentachloride (3.54 g, 17.0 mmol) in phosphorus oxychloride (20.0 mL) was heated under reflux for 1 h. The mixture was concentrated in vacuo to a residue (9.7 g), and the residue was partitioned between 10% NaOH (100 mL) and CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The organic portion was dried (Na<sub>2</sub>SO<sub>4</sub>) then concentrated to give 4.18 g of a tan solid, mp 157.5-158°C. Additional crude product (9.9 g) was obtained from another reaction, mp 156-156.5°C. The two lots were combined then purified by chromatography on silica gel (400 g) column using hexane-EtOAc (4:1) as the eluent. Fractions containing pure product were combined then concentrated to a smaller volume. The solid that separated was collected then dried, yield 6.35 g, mp 155-156°C, literature<sup>15</sup> mp 158-

159°C. A portion (6.14 g) was transmitted to WRAIR on November 17, 1999 (Lot No. 1N3-13-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>Cl</u>	<u>N</u>
Calc'd for $C_{15}H_8ClNO$	70.90	3.21	14.06	5.53
Found	71.01	3.18	13.98	5.52

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  3429, 3063, 2952, 1723, 1692, 1648, 1604, 1560, 1503, 1465, 1453, 1396, 1381, 1331, 1316, 1284, 1249, 1196, 1142, 1104, 1045, 1021, 941, 876, 847, 804, 760, 744, 705, 658, 635, 599, 567, 459, 423  $cm^{-1}$ .

Nuclear Magnetic Resonance (DMSO- $d_6$ )

$^1H$   $\delta$  8.33-8.27 (m, 3H); 7.91-7.77 (m, 4H); 7.57 (t, 1H,  $J=7.5$  Hz).  
 $^{13}C$   $\delta$  159.8, 147.7, 147.1, 144.8, 131.8, 130.1, 129.1, 127.4, 125.7, 124.6, 124.0, 123.5, 123.0, 121.6, 113.0.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>Rf</u>
1. Hexane-EtOAc (7:3)	0.56
2. CH <sub>2</sub> Cl <sub>2</sub> -hexane (1:1)	0.77
3. Ether-hexane (1:1)	0.57

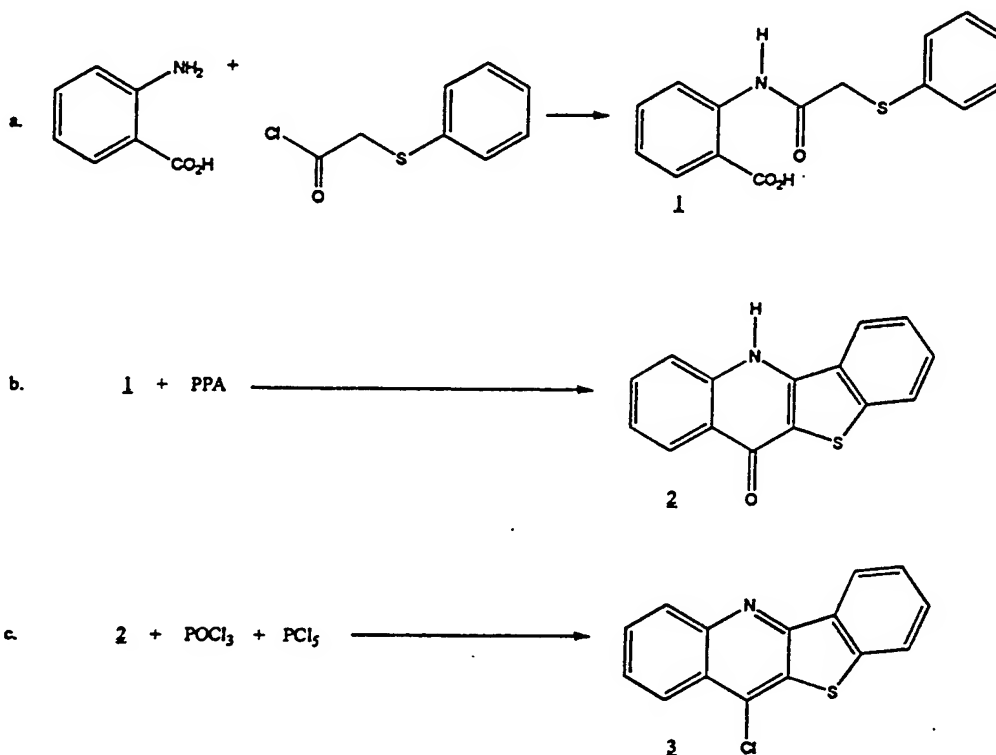
Source of Materials

1. Anthranilic acid	Aldrich Chemical Co., Inc.
2. NaOH	J.T. Baker Chemical Co.
3. Phenoxyacetyl chloride	Aldrich Chemical Co., Inc.
4. HCl	J.T. Baker Chemical Co.
5. EtOAc	J.T. Baker Chemical Co.
6. MgSO <sub>4</sub>	J.T. Baker Chemical Co.
7. Hexane	J.T. Baker Chemical Co.
8. Benzoic acid, 2- [[2-(phenoxy)acetyl]- amino]-	Starks Associates, Inc.
9. PPA	Aldrich Chemical Co., Inc.
10. K <sub>2</sub> CO <sub>3</sub>	J.T. Baker Chemical Co.
11. Benzofuro[3,2-b]- quinoline-11(5H)-one	Starks Associates, Inc.
12. PCl <sub>5</sub>	Aldrich Chemical Co., Inc.
13. POCl <sub>3</sub>	Aldrich Chemical Co., Inc.
14. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.
15. Na <sub>2</sub> SO <sub>4</sub>	J.T. Baker Chemical Co.
16. Silica gel	Merck & Co., Inc.

20. [1]Benzothieno[3,2-b]quinoline, 11-chloro- (3)

The target compound 3 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Benzoic acid, 2-[[2-(phenylthio)acetyl]amino]- (1)

To a cooled (0°C), stirred solution of anthranilic acid (11.0 g, 80.4 mmol) in aq. NaOH (7.0 g, 70 mL) was added, dropwise (over 40 min), (phenylthio)acetyl chloride



(15.16 g, 78.8 mmol). After the addition the mixture was stirred at 0°C for 1 h then neutralized with 5% HCl (5 mL), water was added, and the mixture was extracted with EtOAc (3 x 175 mL). The extracts were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated to a pale-yellow solid (10.3 g), mp 173-177°C; literature<sup>1</sup> mp 164-166°C. Additional product (24.4 g) of similar purity was obtained from another reaction. The aqueous filtrates yielded additional product (5.7 g). The three lots were combined, suspended in 200 mL of hexane-EtOAc (1:1), and stirred at room temperature for 20 min. The solid was collected on a filter, washed with hexane-EtOAc (4:1) (40 mL), then dried to give 37.2 g (70.2% overall yield), mp 158-163°C. The material was suitable for further transformation.

### Spectral Data

#### Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

δ 13.91 (broad s, 1, CO<sub>2</sub>H); 12.60 (broad s, 1, NH); 8.45 (d, 1, J=8.1 Hz); 7.94 (dd, 1, J=8.1 Hz, J=1.8 Hz); 7.48-7.43 (m, 1); 7.39-7.30 (m, 2); 7.28-7.26 (m, 2); 7.17-7.14 (m, 1); 7.09-7.08 (m, 1); 3.93 (s, 2, CH<sub>2</sub>).

#### [1]Benzothieno[3,2-b]quinolin-11(5H)-one (2)

A stirred mixture of 1 (15.0 g, 52.2 mmol) and PPA (99.8 g) was heated at 125-130°C for 1½ h then poured over ice-water (500 mL) and stirred until a uniform suspension was obtained. The mixture was neutralized with solid K<sub>2</sub>CO<sub>3</sub>. The resulting solid was filtered off, washed with hot H<sub>2</sub>O (1.5 L), then dried to give 12.3 g of product 2, mp >300°C. Additional product (18.2 g) was obtained from a large

reaction. The material was suitable for further transformation.

### Spectral Data

#### Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 12.76 (s, 1, NH); 8.54 (dd, 1, J=7.4 Hz, 2.1 Hz); 8.24 (d, 1, J=8.0 Hz); 8.11 (dd, 1, J=7.0 Hz, 1.3 Hz); 7.81-7.74 (m, 2); 7.69-7.64 (m, 2); 7.42-7.37 (m, 1).

#### [1]Benzothieno[3,2-b]quinoline, 11-chloro-(3)

A stirred mixture of 2 (12.3 g, 48.9 mmol) and phosphorous pentachloride (10.2 g, 49 mmol) in phosphorous oxychloride (60 mL) was heated under reflux for 20 min. The mixture was concentrated in vacuo, and the residue was partitioned between 10% NaOH (350 mL) and chloroform (1170 mL). The organic portion was dried (Na<sub>2</sub>SO<sub>4</sub>) then concentrated to a light brown solid (11.7 g). Additional product (18.2 g) of similar purity was obtained from another reaction. The two lots were combined, heated in hexane-EtOAc (4:1) (~2 L) then filtered. The filtrate was chromatographed on a column of SiO<sub>2</sub> (1 kg) using hexane-EtOAc (4:1) as the eluent. Fractions containing pure product were combined then concentrated in vacuo to give 20.5 g of yellow solid. The solid was recrystallized from

EtOAc (425 mL) and hexane (~500 mL); yield 13.0 g, mp 159.5°C, literature<sup>15</sup> mp 158-159°C. A portion (12.9 g) was transmitted to WRAIR on November 17, 1999 (Lot No. 1N3-24-1).

Analysis:

	<u>C</u>	<u>H</u>	<u>Cl</u>	<u>N</u>	<u>S</u>
Calc'd for C <sub>15</sub> H <sub>8</sub> ClNS	66.79	2.99	13.14	5.19	11.89
Found	66.86	3.03	12.97	5.10	12.08

Spectral Data

FT-Infrared (KBr pellet)

ν 3057, 1592, 1565, 1545, 1487, 1453, 1375, 1339, 1321, 1298, 1245, 1164, 1105, 1020, 996, 949, 902, 840, 798, 784, 764, 737, 686, 652, 603, 449.

Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

δ 8.53 (d, 1, J=7.6 Hz); 8.30 (d, 2, J=8.2 Hz); 8.17 (d, 1, J=7.8 Hz); 7.94 (t, 1, J=6.7 Hz, 8.2 Hz); 7.84 (d, 1, J=8.0 Hz); 7.76 (t, 1, J=7.7 Hz); 7.66 (t, 1, J=7.6 Hz).

<sup>13</sup>C δ 165.5, 154.3, 147.8, 141.2, 135.4, 135.1, 130.8, 130.3, 129.9, 127.6, 125.9, 125.0, 124.8, 123.6, 123.3.

Thin Layer Chromatography

EM precoated, glass TLC plates. Silica gel 60F-254,  
0.25 x 50 x 100 mm. Detection: UV light.

<u>Eluent</u>	<u>Rf</u>
1. Hexane-EtOAc (7:3)	0.70
2. CH <sub>2</sub> Cl <sub>2</sub> -hexane (1:1)	0.86
3. Ether-hexane (1:1)	0.75

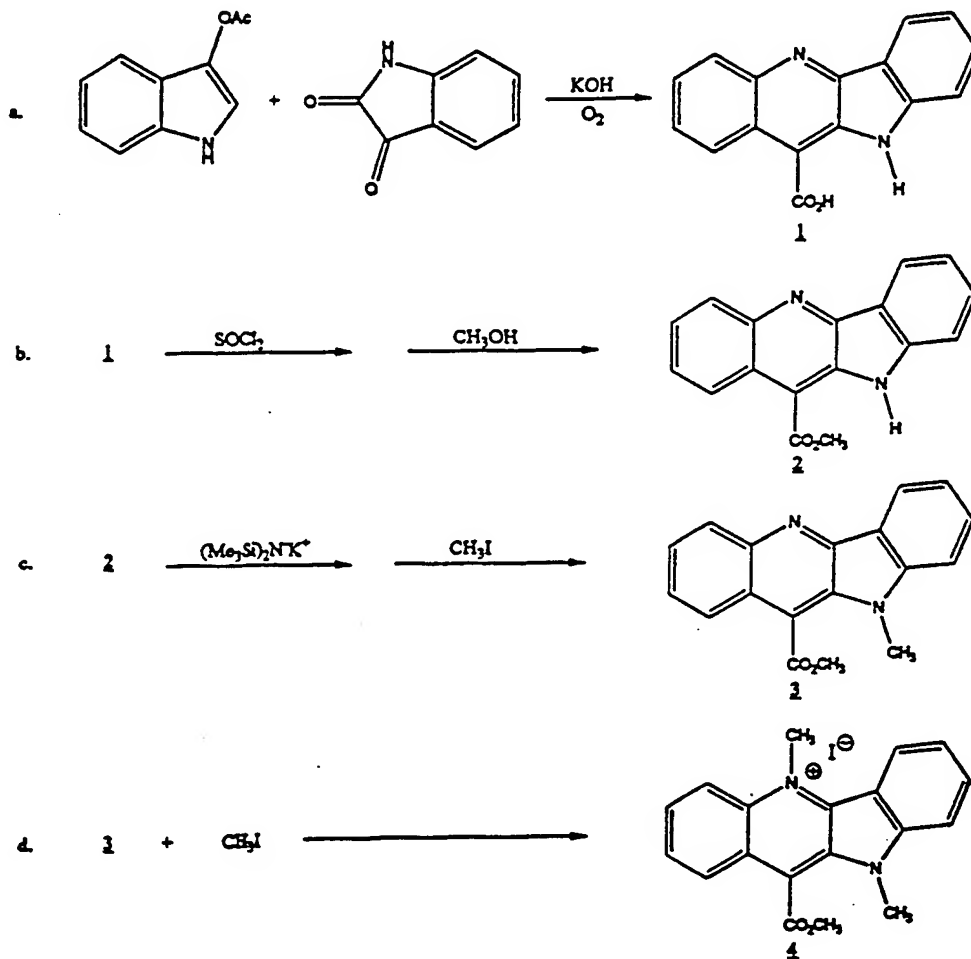
Source of Materials

1. Anthranilic acid	Aldrich Chemical Co., Inc.
2. NaOH	J.T. Baker Chemical Co.
3. (Phenylthio)acetyl chloride	Aldrich Chemical Co., Inc.
4. HCl	J.T. Baker Chemical Co.
5. EtOAc	J.T. Baker Chemical Co.
6. MgSO <sub>4</sub>	J.T. Baker Chemical Co.
7. Hexane	J.T. Baker Chemical Co.
8. Benzoic acid, 2- [[2- (phenylthio)acetyl]- amino] -	Starks Associates, Inc.
9. PPA	Aldrich Chemical Co., Inc.
10. K <sub>2</sub> CO <sub>3</sub>	J.T. Baker Chemical Co.
11. [1]Benzothieno[3,2-b]- quinoline-11(5H)-one	Starks Associates, Inc.
12. PCl <sub>5</sub>	Aldrich Chemical Co., Inc.
13. POCl <sub>3</sub>	Aldrich Chemical Co., Inc.
14. CH <sub>2</sub> Cl <sub>2</sub>	J.T. Baker Chemical Co.

- |     |                          |                         |
|-----|--------------------------|-------------------------|
| 15. | $\text{Na}_2\text{SO}_4$ | J.T. Baker Chemical Co. |
| 16. | Silica gel               | Merck & Co., Inc.       |

21. 10H-Quindolinium, 11-(methoxycarbonyl)-5,10-dimethyl-, iodide (4)

The target compound 4 was prepared by the following sequence of reactions.



Experimental

10H-Quindoline-11-carboxylic acid (1)

To a 500 mL three-neck flask was added 3-indolyl acetate (5.2 g of 97%, 28.5 mmol), then a cooled solution of isatin (4.4 g of 98%, 28.8 mmol) in aqueous KOH (29.5 g of 85%, 447 mmol) in H<sub>2</sub>O (115 mL), under an argon

atmosphere. The mixture was stirred under argon for 3 days, then diluted with H<sub>2</sub>O (50 mL). The mixture was heated to 75-80°C, and oxygen was bubbled through the stirred mixture for 20 min. The mixture was filtered while hot, the filtrate was cooled to ~20°C, then diluted with sufficient ethanol to dissolve the formed precipitate (~75 mL). Concentrated HCl (33 mL) was added to adjust the pH of the mixture to pH=4. The precipitate was collected, washed with hot H<sub>2</sub>O (100 mL), EtOH (100 mL), then dried (Na<sub>2</sub>SO<sub>4</sub>), to give 6.4 g of yellow solid, mp 331-336°C; literature<sup>16</sup> mp 330-337°C. Additional product (15.8 g) was obtained from a larger reaction.

### Spectral Data

#### Nuclear Magnetic Resonance (DMSO-d<sub>6</sub>)

<sup>1</sup>H δ 11.38 (s, 1H, NH); 9.07 (d, J=8.4 Hz, 1H); 8.24 (d, J=8.4 Hz, 1H); 7.75 (d, J=8.1 Hz, 1H); 7.67-7.60 (m, 3H); 7.30 (t, J=7.7 Hz, 1H).

#### 10H-Quindoline-11-carboxylic acid, methyl ester (2)

A suspension of 1 (95 g, 36.2 mmol) in thionyl chloride (119.1 g, 73.0 mL, 1.00 mol) was heated under reflux for 2 h then cooled. Excess thionyl chloride was removed in vacuo, and the residue was diluted with anhydrous MeOH (225 mL) then heated, under reflux, for 1 h. The solvent was removed in vacuo, and the residue was basified then extracted with EtOAc (2 x 200 mL). The extracts were combined, washed with H<sub>2</sub>O (2 x 100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), then concentrated in vacuo. The residue was recrystallized from hexane, yield 6.3 g (63%); mp 150-151°C, literature<sup>15</sup> mp 152-153.5°C. Additional product (5.9

g) was obtained from other reactions. The material was suitable for further transformation.

### Spectral Data

#### Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H δ 9.75 (s, 1H, NH); 9.08 (dd, J=7.7 Hz); 2.6 Hz, 1H); 8.51 (d, J=7.7 Hz, 1H); 8.39 (dd, J=7.0 Hz, 2.2 Hz, 1H); 7.74-7.61 (m, 1H); 7.48 (d, J=8.1 Hz, 1H); 7.38 (t, J=7.7 Hz, 1H); 4.19 (s, 3H, CH<sub>3</sub>).

#### 10H-Quindoline-11-carboxylic acid, 10-methyl-, methyl ester (3)

To a cooled (0°C) solution of 2 (8.1 g, 29.3 mmol) in THF (405 mL) was added a 0.5M solution of potassium bis(trimethylsilyl)amide in toluene (81.4 mL, 40.7 mmol). A red solution was obtained. After 15 min methyl iodide (2.41 mL, 5.5 g, 38.7 mmol) was added, and the mixture was stirred at RT overnight, then quenched with aqueous saturated ammonium chloride (200 mL). The mixture was extracted with EtOAc (3 x 200 mL). The extracts were combined, then concentrated in vacuo to a residue (9.1 g). The residue was adsorbed on silica gel (91 g) then chromatographed on a column of silica gel (1 kg) using hexane-EtOAc (7:3) as the eluent. Fractions containing pure product (as determined by TLC) were concentrated in vacuo to a bright yellow crystalline mass (7.2 g, 84.7%). mp 127°C; literature<sup>15</sup> mp 127°C. Additional product (3.0 g) was obtained from another reaction. The material was suitable for further transformation.



# Spectral Data

## Nuclear Magnetic Resonance (400 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H δ 8.56 (d, J=7.7 Hz, 1H); 8.37 (d, J=8.4 Hz, 1H); 8.01 (d, J=8.4 Hz, 1H); 7.72 (t, J=7.0, 8.4 Hz, 1H); 7.69 (t, J=7.9, 8.1 Hz, 1H); 7.64 (t, J=7.3, 7.7 Hz, 1H); 7.44 (d, J=8.1 Hz, 1 H); 7.39 (t, J=7.7, 7.3 Hz, 1H); 4.21 (s, 3H, OCH<sub>3</sub>); 3.85 (s, 3H, NCH<sub>3</sub>).

10H-Quindolinium, 11-(methoxycarbonyl)-5,10-dimethyl-,  
iodide (4)

---

A solution of 3 (3.0 g, 10.3 mmol) in CH<sub>3</sub>I (50 mL, 119 g, 803 mmol) was stirred at RT for 48 h. The solid that separated was collected, washed with ether (into a separate flask) then dried, yield 0.8 g. The filtrate was stirred at RT for 48 h to give additional product (0.7 g). The filtrate and washings were combined then concentrated to a residue (2.1 g). The residue was triturated with ether to give additional product (0.6 g), total yield (2.1 g). The ether filtrate was concentrated in vacuo to a residue (1.5 g). This material was dissolved in CH<sub>3</sub>I (25 mL) and heated under reflux for 24 h. The solid (0.9 g) was collected. Concentration of filtrate gave 0.9 g of residue, which was again methylated to give additional product (0.2 g), total yield 3.2 g (72.7%). The solid was dissolved in CH<sub>3</sub>OH (450 mL), the solution was filtered, then diluted with ether (1350 mL). The solid was collected then dried, yield, 2.4 g (75% recovery). Additional product (1.1 g) was obtained from another reaction. The two lots were combined, thoroughly blended then characterized as a single lot, mp 200-201°C(d), literature<sup>15</sup> mp 257-257.5°C. A portion (3.4 g) was transmitted to WRAIR on December 29, 1999 (Lot No. 2N3-19-2).

Analysis:

	<u>C</u>	<u>H</u>	<u>N</u>	<u>I</u>
Calc'd for $C_{19}H_{17}N_2O_2I$	52.79	3.96	6.48	29.36
Found	52.91	3.96	6.46	29.49

Spectral Data

FT-Infrared (KBr pellet)

$\nu$  3008, 2948, 1735, 1627, 1596, 1514, 1492, 1487, 1472, 1412, 1365, 1347, 1327, 1292, 1263, 1237, 1198, 1168, 1149, 1072, 1047, 1032, 1011, 839, 788, 759, 709, 506, 463, 424  $cm^{-1}$ .

Nuclear Magnetic Resonance (DMSO- $d_6$ )

$^1H$   $\delta$  8.92 (d, 1,  $J=9.2$  Hz, 1H); 8.91 (d,  $J=8.1$  Hz, 1H); 8.34 (d,  $J=8.4$  Hz, 1H); 8.25 (ddd,  $J=8.5$  Hz,  $J=7.0$  Hz,  $J=1.1$  Hz, 1H); 8.12-8.03 (m, 3H); 7.62 (ddd,  $J=8.4$  Hz,  $J=6.2$  Hz,  $J=1.8$  Hz, 1H); 5.10 (s, 3H); 4.33 (s, 3H); 4.01 (s, 3H).

$^{13}C$   $\delta$  166.2, 148.4, 141.8, 136.4, 133.9, 131.3, 130.1, 128.2, 127.1, 123.6, 120.0, 114.9, 113.0, 56.0, 47.9, 42.5, 41.7, 40.0, 32.3.

Mass (Electrospray ionization)

$m/z$  304.9  $[M]^+$

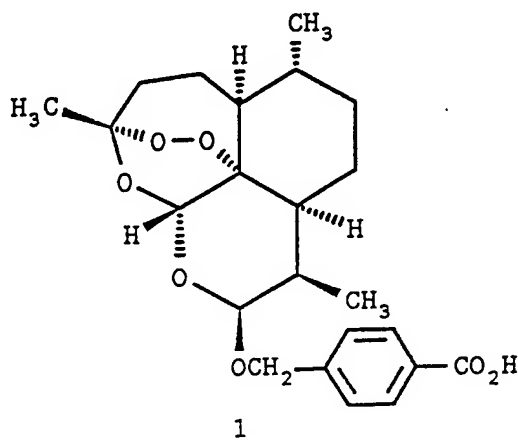
Source of Materials

- |     |   |                            |
|-----|---|----------------------------|
| 1.  | 3-Indolyl acetate   | Aldrich Chemical Co., Inc. |
| 2.  | Isatin  | Aldrich Chemical Co., Inc. |
| 3.  | KOH   | Aldrich Chemical Co., Inc. |
| 4.  | Ethanol   | Aaper Alcohol & Chem. Co.  |
| 5.  | HCl   | J.T. Baker Chemical Co.    |
| 6.  | 10H-Quindoline-11-carboxylic acid                           | Starks Associates, Inc.    |
| 7.  | Thionyl chloride  | Aldrich Chemical Co., Inc. |
| 8.  | MeOH  | J.T. Baker Chemical Co.    |
| 9.  | EtOAc   | J.T. Baker Chemical Co.    |
| 10. | Hexane  | J.T. Baker Chemical Co.    |
| 11. | 10H-Quindoline-11-carboxylic acid, methyl ester             | Starks Associates, Inc.    |
| 12. | THF   |                            |
| 13. | Potassium bis(trimethylsilyl)amide, 0.5M in toluene         | Aldrich Chemical Co., Inc. |
| 14. | NH <sub>4</sub> Cl  | Aldrich Chemical Co., Inc. |
| 15. | Silica gel  | EM Laboratories            |
| 16. | 10H-Quindoline-11-carboxylic acid, 10-methyl-, methyl ester | Starks Associates, Inc.    |
| 17. | CH <sub>3</sub> I   | Aldrich Chemical Co., Inc. |
| 18. | Ether   | Fisher Scientific, Inc.    |

## Key Research Accomplishments

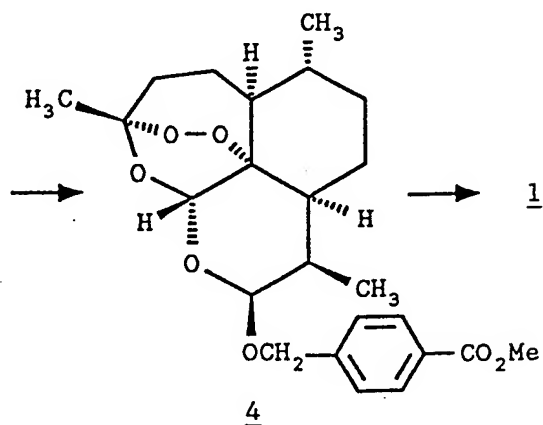
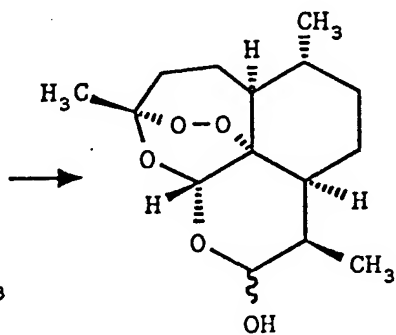
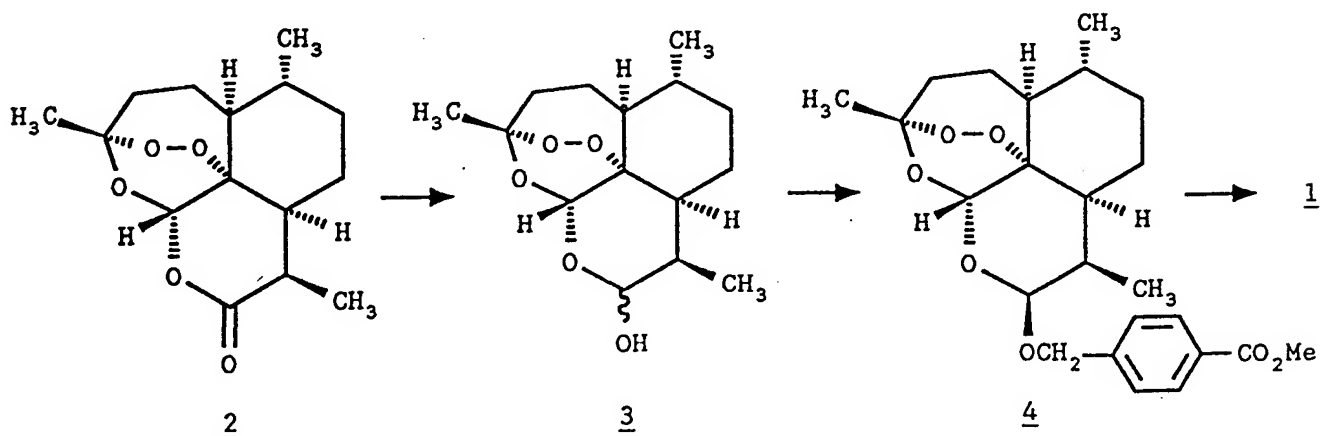
1.  $\beta$ -Artelinic acid (cGMP) (1)

The first assignment of the new contract (DAMD17-99-D-0005) was the preparation of additional  $\beta$ -artelinic acid (cGMP) (1).



The first portion of target material (1) was prepared under our previous contract (DAMD17-93-C-3003) and 3900 g (Lot No. NJ33-113-1) was transmitted to WRAIR on January 26, 1999.

The remainder of the material 1 was prepared under the Task Order 0001 in a similar manner by the following sequence of reactions:



Commercially available artemisinin (2) was reduced with sodium borohydride to give a mixture of epimeric dihydroartemisinins (3) in 84% yield. This was not purified, but immediately reacted with methyl 4-hydroxy-methylbenzoate in the presence of boron trifluoride diethyl etherate to give the methyl ester of artelinic acid (4) in 67% yield. Hydrolysis of the ester with methanolic KOH at RT gave the target compound 1 in 86% yield. A portion (1871.0 g) was transmitted to WRAIR on June 7, 1999 (Lot No. 1N1-38-1).

In our preliminary (non-cGMP) efforts, each step of the reaction sequence was examined for potential optimization. The original literature procedure<sup>1a,1b</sup> called for a large excess of sodium borohydride in the first step of the sequence. We found that the molar ratio of sodium borohydride to artemisinin 2 could be decreased without affecting the yield of the product 3. Similarly, the amount of methanol was decreased by 50% without a decrease in product yield. Details of these changes are shown in Table 1.

TABLE 1

<u>2</u>	<u>Amounts used</u>		<u>Molar Ratio of</u>		<u>Yield of 3</u>		<u>Comments</u>
	<u>NaBH<sub>4</sub></u>	<u>CH<sub>3</sub>OH</u>	<u>2</u>	<u>: NaBH<sub>4</sub></u>	<u>grams</u>	<u>%</u>	
5 g	5 g	200 mL	1	7.5	3.6	70.6	Lit. method
5 g	5 g	200 mL	1	7.5	4.4	86.8	New work-up
5 g	2.5 g	200 mL	1	3.7	4.6	90.0	
5 g	1.75 g	200 mL	1	2.5	4.5	88.8	Longer time required
5 g	2 g	100 mL	1	2.9	4.6	90.0	Method to be used in the scale-up
25 g	10 g	500 mL	1	2.9	23.2	91.7	

In the second step, a series of reaction conditions were explored which varied the ratio of dihydroartemisinin 3 to methyl 4-hydroxymethylbenzoate. The results are summarized on the following page in Table 2.

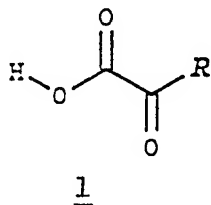
TABLE 2

<u>3</u>	<u>Amounts used</u>		<u>Molar Ratio of</u>		<u>Yield of 4</u>		<u>Comment</u>
	<u>methyl ester</u>	<u>ether</u>	<u>3 : methyl ester</u>		<u>gram</u>	<u>%</u>	
4.1 g	8 g	450 mL	1	3.5	5.9	98.3	lit. method
21.4 g	37 g	2200 mL	1	3	32.4	100.0	
2.0 g	2.33 g	200 mL	1	2	2.5	83.3	
2.0 g	2.33 g	100 mL	1	2	2.9	96.7	
2.0 g	1.74 g	50 mL	1	1.5	2.3	76.4	
2.0 g	2.33 g	50 mL	1	2	3.0	100.0	
2.0 g	1.74 g	50 mL	1	1.5	3.1	100.0	method to be used in scale-u

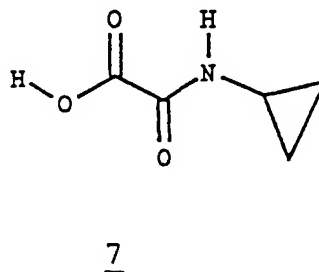
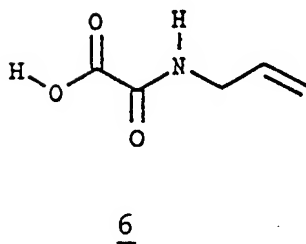
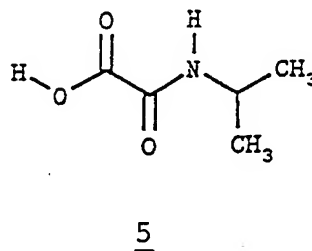
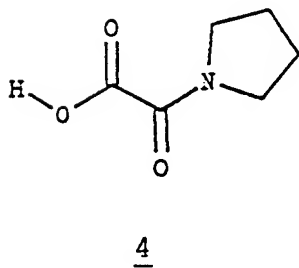
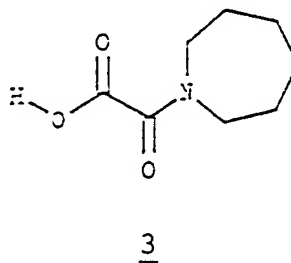
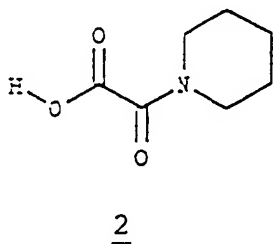
The conditions described in the final entry of Table 2 were used in scale-up. The molar ratio of dihydroartemisinin 3 to methyl 4-hydroxymethylbenzoate was able to be decreased from 1:3½ to 1:1½, thus using only 43% of the amount of ester which would be required of the literature<sup>2</sup> method. The amount of ether used in this step could be decreased by over 75% with no change in product yield, so this modification was utilized as well. We found that the original conditions of hydrolysis in the final step (conversion of 4 to 1) to be satisfactory, so they remained unchanged.

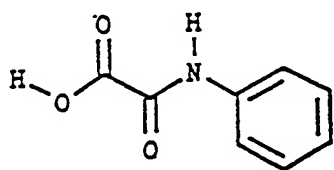
## 2. Oxamic acids

The second task order assignment consisted of the synthesis of oxamic acids 1 where R is generally the residue of a primary or secondary amine.

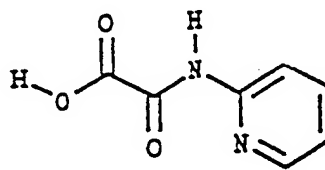


Sixteen of these acids (2 - 17) have been completed and submitted to WRAIR.

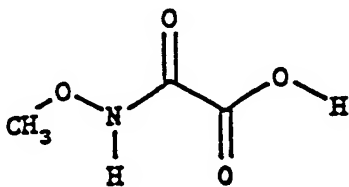




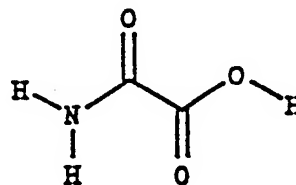
8



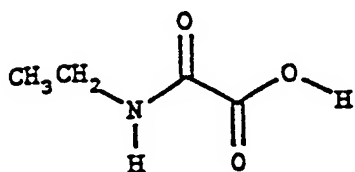
9



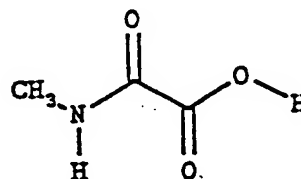
10



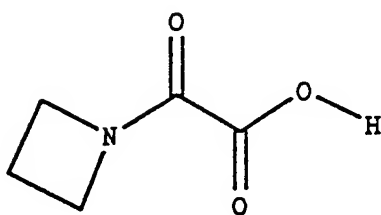
11



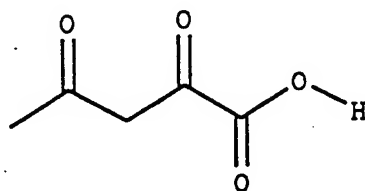
12



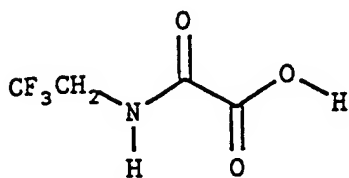
13



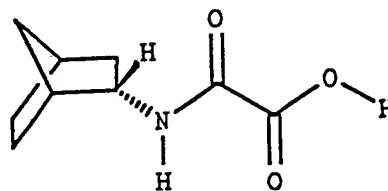
14



15



16

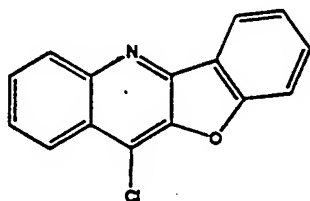


17

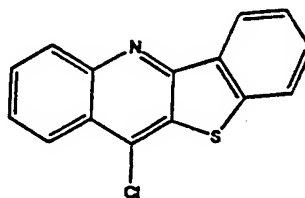


## 2. Heterocycles

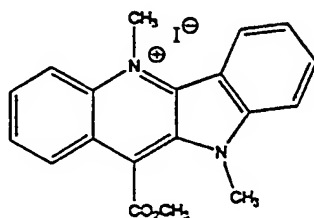
The third task order consisted of the synthesis of three heterocycles shown below:



1

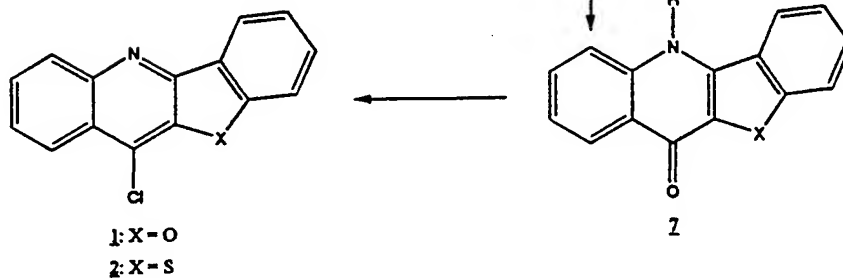
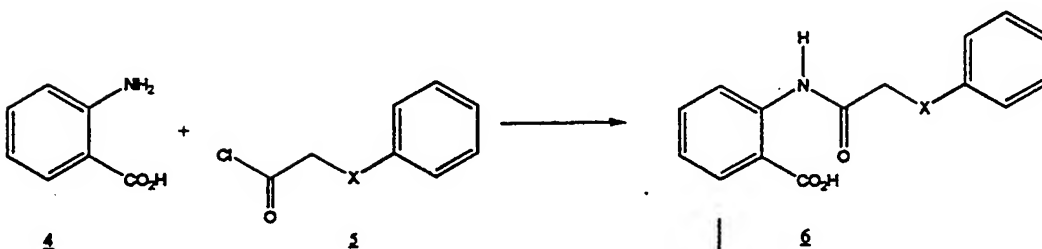


2

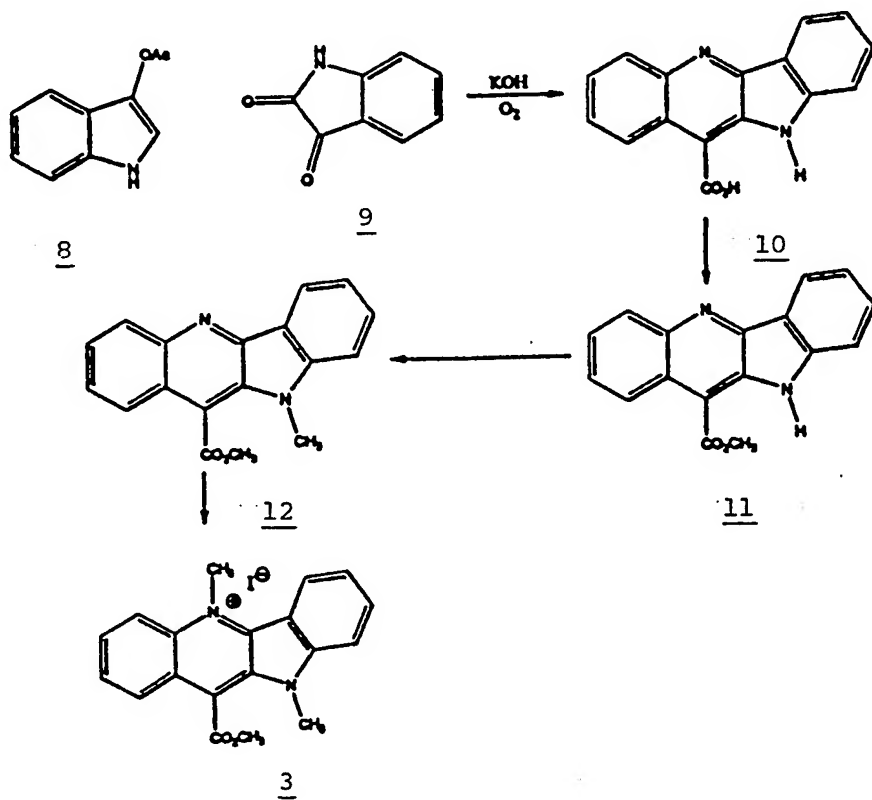


3

The first two materials have been prepared as shown below:



The third material was prepared as shown below:



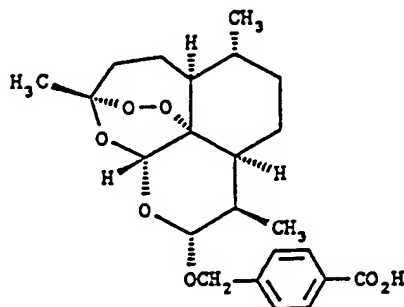
Condensation of 3-indolyl acetate (8) with isatin (9) in base gave quindolinecarboxylic acid (10). Reaction of 10 with thionyl chloride gave the acid chloride which with methanol gave the methyl ester 11. Product 11 was methylated with CH<sub>3</sub>I using 0.5M solution of bis(trimethylsilyl)amide in toluene as the base. Reaction with CH<sub>3</sub>I gave the target material 3.

## Conclusions

CUMULATIVE LIST OF REQUESTED COMPOUNDS DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM FEBRUARY 15, 1999 TO FEBRUARY 14, 2000

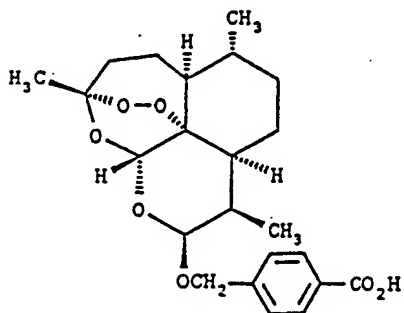
The previous Cumulative List covering the period from December 1, 1992 to March 31, 1999 may be found in Starks Associates, Inc. Final Report dated May 1999, page 48, Contract No. DAMD17-93-C-3003. The list covering the period from March 15, 1989 to November 30, 1992 may be found in Starks Associates, Inc. Final Report dated November 30, 1992, page 35, Contract No. DAMD17-89-C-9058. The list covering the period from September 15, 1983 to March 14, 1989 may be found in Starks Associates, Inc. Final Report dated March 14, 1989, page 55, Contract DAMD17-83-C-3206. The list covering the period from September 29, 1979 to September 14, 1983 may be found in Starks Associates, Inc. Final Summary Report dated September 1983, page 56, Contract No. DAMD17-79-C-9170. The list covering the period from July 1, 1973 to September 28, 1979 may be found in Starks Associates, Inc. Final Summary Report dated September 1979, page 82, Contract No. DAMD17-73-C-3159. The list covering the period from July 1, 1965 to June 30, 1973 may be found in Starks Associates, Inc. Final Summary Report dated June 1973, page 54, Contract No. DA49-193-MD-2751.

<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
---------------------------------	-----------------	---------------	------------	------------	-------------------------------------

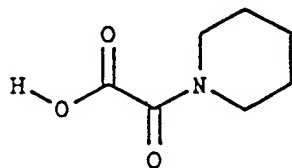


1257	$\alpha$ -Artelinic acid	438.6 g		282644	137
------	--------------------------	---------	--	--------	-----

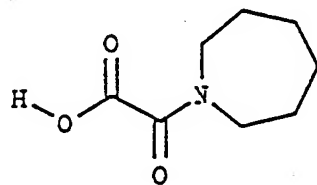
<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
---------------------------------	-----------------	---------------	------------	------------	-------------------------------------



1258	$\beta$ -Artelinic acid, hemihydrate (cGMP)	1871.0 g		255663	137
------	--	----------	--	--------	-----

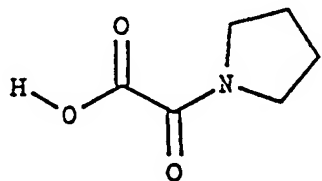


1259	1-Piperidineacetic acid, .alpha.-oxo-	2.1 g			137
------	--	-------	--	--	-----

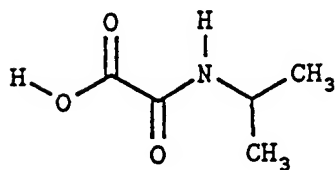


1260	1H-Azepine-1- glyoxylic acid, hexahydro-	3.4 g			137
------	--	-------	--	--	-----

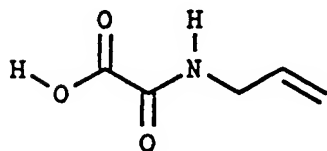
<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
---------------------------------	-----------------	---------------	------------	------------	-------------------------------------



1261	1-Pyrrolidine- acetic acid, .alpha.-oxo-	1.8 g			137
------	--	-------	--	--	-----

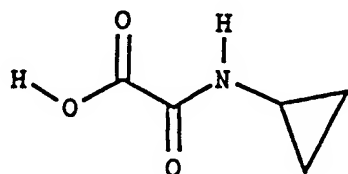


1262	Acetic acid, [(1-methylethyl)- amino]oxo-	2.7 g			137
------	---	-------	--	--	-----

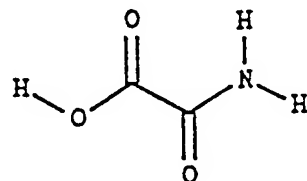


1263	Acetic acid, oxo- (2-propenylamino)-	2.5 g			137
------	---	-------	--	--	-----

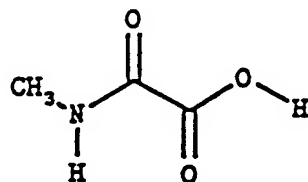
<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
---------------------------------	-----------------	---------------	------------	------------	-------------------------------------



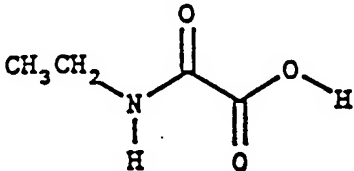
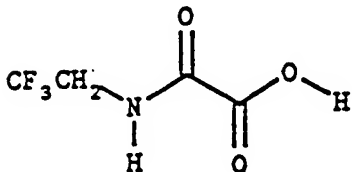
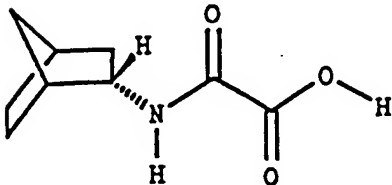
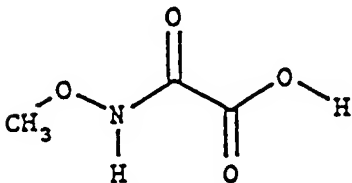
1264	Acetic acid, (cyclopropyl- amino) oxo-	0.5 g			137
------	--	-------	--	--	-----

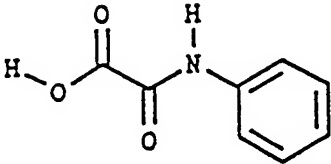
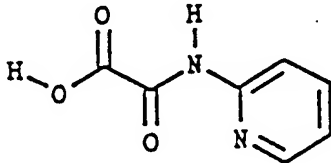
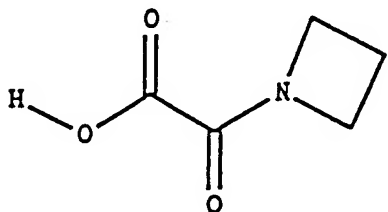
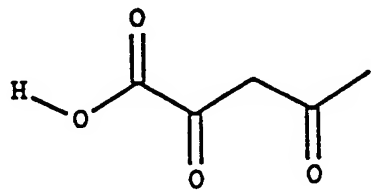


1265	Acetic acid, aminooxo-	2.0 g			138
------	---------------------------	-------	--	--	-----

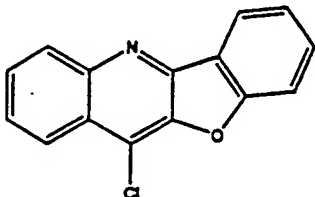
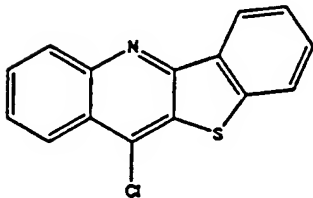
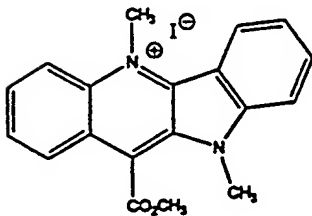


1266	Acetic acid, (methylamino) oxo-	0.75 g			138
------	------------------------------------	--------	--	--	-----

<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
					
1267	Acetic acid, (ethylamino)oxo-	1.0 g			138
					
1268	Acetic acid oxo- [(2,2,2-trifluoro- ethyl)amino]-	1.06 g			138
					
1269	Acetic acid, [(bicyclo[2.2.1]- hept-2-yl)amino]- oxo-, endo-	0.88 g			138
					
1270	Acetic acid, (methoxyamino)oxo-	0.988 g			138

<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
					
1271	Acetic acid, oxo- (phenylamino) -	0.856 g			138
					
1272	Acetic acid, oxo(2- pyridinylamino) -	2.3 g			138
					
1273	1-Azetidineacetic acid, .alpha. -oxo-	0.70 g			138
					
1274	Pentanoic acid, 2,4-dioxo-	0.301 g			138



<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
					
1275	Benzofuro [3,2-b] - quinoline, 11-chloro-	6.1 g			138
					
1276	[1] Benzothieno- [3,2-b]quinoline, 11-chloro-	12.9 g			138
					
1277	10H-Quindolinium, 11- (methoxycarbonyl) - 5,10-dimethyl-, iodide	3.4 g			139

References:

1.   a.   R.R. Engle, personal communication (August 13, 1986). Preparation of dihydroartemisinin by A.J. Lin.  
  
      b.   H. A. Musallam, personal communication (August 27, 1988). Preparation of dihydroartemisinin by B. Venugopalin and A. Brossi.
2.   H. A. Musallam, personal communication (April 29, 1987).
3.   Coppa, F.; Fontana, F.; Lazzarini, E.; Minisci, F. *Heterocycles*, 1993, 36(12), 2687-2696. This describes a general procedure for the preparation of monoamides of oxalic acid including the title compound. No physical constants are given.
4.   Wallach, O.; Lehmann, F. *Justus Liebigs Ann. Chem.* 1887, 247, 236-260.
5.   Pirrung, M.C.; Han, H.; Ludwig, R.T. *J. Org. Chem.* 1994, 59, 2430-2436.
6.   Gluud, W. *J. Chem. Soc.* 1913, 940-946.
7.   Wolfs, I.; Desseyn, H.O. *Spectrochim. Acta*, Part A, 1996, 52A(11), 1521-1528.
8.   Catalog Handbook of Fine Chemicals 1998-1999, Aldrich Chemical Co., Inc., p. 1256.
9.   The compound is unknown to the chemical literature.

10. Byeroft, B.N; Gledhill, L.; Shute R.E.; Williams, P.  
*J. Chem. Soc., Chem. Commun.* **1988**, 1610-1612. No  
physical constants are given.
11. CRC Handbook of Chemistry & Physics, 58<sup>th</sup> Edition,  
1977-1978, page C409.
12. Ohta, V.N; Masaki, M. *Bull. Chem. Soc. Japan*, **1960**,  
33, 1392-1394.
13. Edmond, J.D.; Johnston, R.G.; Kidd, D.; Rylance, H.J.;  
Sommerville, R.G. *Brit. J. Pharmac. Chemotherap.* **1966**,  
27, 415-425.
14. CRC Handbook of Chemistry and Physics, 58<sup>th</sup> Edition,  
1977-1978, page C418.
15. COL J. Scovill, personal communication, May 26, 1999.
16. Holt, J.S.; Petrov, V. *J. Chem. Soc.* **1947**, 607-611.

## Appendices

### ACKNOWLEDGEMENT

The following technical personnel were assigned to syntheses which have been requested by Walter Reed Army Institute of Research. Dr. J.F. Novotny (Supervisor), Dr. S.K. Chadda, and Mr. D.R. Saunders.

We would like to acknowledge the technical help of COL J. Scovill and CPT M.J. Novak.

**DISTRIBUTION**

3 Copies to

Commander  
US Army Medical Research and  
Materiel Command  
Attn: MCMR-RMI-S  
504 Scott Street  
Fort Detrick MD 21702-5012